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CONTENTS

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RELATION OF A LARGE SOIL–BORNE SPORE TO PHYCOMYCETOUS MYCORRHIZAL INFECTIONS

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(WITH 18 FIGURES)

The so-called "vesicular-arbuscular" or "phycomycetous" mycor-rhizal fungus has been described by many investigators as occurring on a great variety of wild and cultivated plants in widely scattered areas of the world. This type of mycorrhizal fungus can be recognized by the irregular coenocytic hyphae which penetrate directly through the cells of the outer cortex of the root. Coils and continuous loops are frequently produced by penetrating hyphae. When hyphae reach the inner cortex, their growth is mainly intercellular with haustorium-like structures called arbuscules forming within the cells. Terminal bodies called vesicles often form in the intercellular spaces in the outer cortex.

In Illinois, the feeder-root system of red clover, *Trifolium pratense* L., is short-lived and goes through cycles of loss and regeneration similar to that described by Jones (**8**) for alfalfa, *Medicago sativa* L. The mycor-rhizal fungus is one of the most conspicuous fungi associated with the young rootlets of red clover. All attempts to isolate this fungus directly from roots have failed, and consequently it has been impossible to deter-mine the effect of the fungus. Diverse opinions have been expressed by other workers as to the relation of the fungus to the host. Some plant

pathologists have taken the view that it is a pathogen, while others believe that it is a symbiont. Since investigators have not been successful in isolating and testing the organism in pure culture, there is little evidence for either point of view.

This type of mycorrhiza appears to be a normal component of the roots of many agricultural crops. After examination of plants from numerous alfalfa and pea, *Pisum sativum* L., fields in Michigan, Wisconsin, Utah, Idaho, and Montana, Jones (7) reported that all fields appeared infested to some degree. He concluded that not many leguminous plants which are potential hosts reach maturity without becoming invaded. Many published accounts list large numbers of species of plants in which this type of mycorrhizal fungus has been observed.

Because of the widespread occurrence of this fungus within the roots of plants, knowledge of the relationship between the fungus and host appears necessary for a clearer understanding of both normal and pathologic root development. Determination of the life history and physiology of the fungus may aid in the development of techniques for solution of the problem of host-parasite relations. This paper includes one phase of a study to determine the relationship of the soil-borne spore-like bodies to the mycorrhizal infection of the root.

Comprehensive literature reviews concerned with this type of mycorrhiza have been published recently by Kelley (9) and Harley (6), consequently only a few papers will be discussed here. The first name applied to a member of this group was *Rhizophagus populinus* Dangeard (3, 4). Dangeard's description was based only on the appearance of the fungus within the root. Galland (5) and other investigators have demonstrated considerable variation in the appearance of this type of mycorrhiza from one group of plants to another. However, there are no data available to determine adequately whether one or more species is involved. The described variation could be due to different species, variability within species, or to a response of one species to different hosts. The name *Rhizophagus teae* (Zimm.) Butler is commonly used for the form on tea. However, only vesicles were described in the exceedingly brief original description (14), and it is doubtful if they could be distinguished from those described for *Rhizophagus populinus*. Until consistent morphological differences are shown to exist between isolates of known purity, it would be better to refer fungi of this general type to either *Rhizophagus populinus* or to avoid the use of specific names.

The systematic position of this type of mycorrhizal fungus is also uncertain. Butler (2) described vesicles produced outside of roots and

noted their resemblance to the so-called chlamydospores of the Endogonaceae. Peyronel (**11**) reported that species of *Endogone* were responsible for the endotropic mycorrhiza of some plants. Mosse (**10**) recently reported fructifications of the *Endogone* type attached to strawberry roots containing the mycorrhizal fungus. She reported "typical mycorrhizal infection" when these fruiting bodies were added to sterile soil with strawberry seedlings.

Many workers have attempted to isolate this fungus, and a few have reported success; however, Harley (**6**) states that in no case reported have the isolated fungi, when inoculated on sterile seedlings, formed arbuscules or vesicles which have been convincingly described or figured. Barrett (**1**) succeeded in obtaining pure cultures from mycelium and external vesicles of the type described by Butler (**2**). However, he has not yet reported successful inoculations with these cultures.

MATERIALS AND METHODS

Spores used in this study were obtained from two soil sources: 1) A field on the Illinois Agricultural Experiment Station, Agronomy South Farm, Urbana, Illinois, where Kenland red clover had been growing continuously for three years. Soil was carefully collected from near clover roots; however, the presence of grass weeds made it uncertain that all spores obtained from these samples were produced on clover; and 2) the Illinois Agricultural Experiment Station, Morrow Plots, located on the campus of the University. Soil was collected from near the roots of corn, *Zea mays* L., plants in a plot where corn has been grown continuously without treatment since 1876. All weeds are carefully removed from the plot each year and it may be assumed that spores obtained from this soil were produced on corn.

Spore-like bodies were removed from soil in quantity by the process of wet sieving and decanting as used for recovering cysts of *Heterodera* from non-desiccated samples. Approximately 250 cc of soil collected from near plant roots was suspended in a liter or more of water. Soil particles were allowed to settle for a few seconds and the liquid decanted through a No. 18 sieve (1 mm openings). The suspension that passed through this sieve was retained and poured through a No. 60 sieve (0.25 mm openings). The material retained on the No. 60 sieve was then washed under the tap, transferred to a Petri dish, and examined under the low power of a stereoscopic microscope. Spores were separated from the remaining debris by means of a transfer needle.

Roots were cleared and stained by boiling for about 10 minutes in a

saturated solution of chloral hydrate containing 0.01% acid fuchsin and examined in clear lactophenol.

SOIL-BORNE SPORE-LIKE BODIES

The nature and relation of the various spore-like bodies associated with phycomycetous mycorrhizal infections is not clear; therefore, it seems best at present to avoid the use of spore names. To facilitate discussion, the bodies that are considered in this paper are designated in the following manner.

Type A spores (FIG. 1) represent the vesicles described by various workers and considered by Butler (2) to be similar to the chlamydospores of the Endogonaceae. They are borne in irregular groups, and have not been described as exceeding 200 μ in diameter. Hyphae are attached directly to the spore, without a bulbous swelling, and the spores are not separated from the hyphae by a cross wall. Since the internal and external vesicles appear to be comparable, they are both considered as type A spores. Type A spores usually do not germinate. However, attached hyphae will continue to grow and hyphae also grow from the broken ends of old hyphae.

Type B spores (FIGS. 2, 3) apparently have not been previously described. They are borne singly and are bright yellow, one-celled, with a thin outer and thick inner wall, spherical, ellipsoidal, or irregular in shape, filled with oil droplets and measuring 183–500 × 291–812 μ. The attached hypha enlarges into a distinct bulbous swelling, 41–51 μ in diameter, at the base of the spore. An antheridium-like tube extends from the bulbous swelling to the base of the spore. There is often a cross wall in the tube about midway between the bulbous swelling and the spore, and there is usually a septum in the attached hypha just below the bulbous swelling. The spores tend to be spherical but are often highly irregular in size and shape (FIG. 3). The shape of the spore is probably determined to some extent by the available space between soil particles. B spores germinate by the production of germ tubes from the base of the spore.

Type C spores (FIG. 4) apparently have not been previously described. They occur in compact clusters of from 1–16. They are yellow, one-celled, filled with oil droplets, spherical to slightly clavate or occasionally irregular in shape, with the apex somewhat flattened and echinulate, and are 22–37 × 20–34 μ in size. They are not cut off from the hyphae by septa. Germination has not been observed. Type C spores form on hyphae growing from B spores. A short thick lateral branch
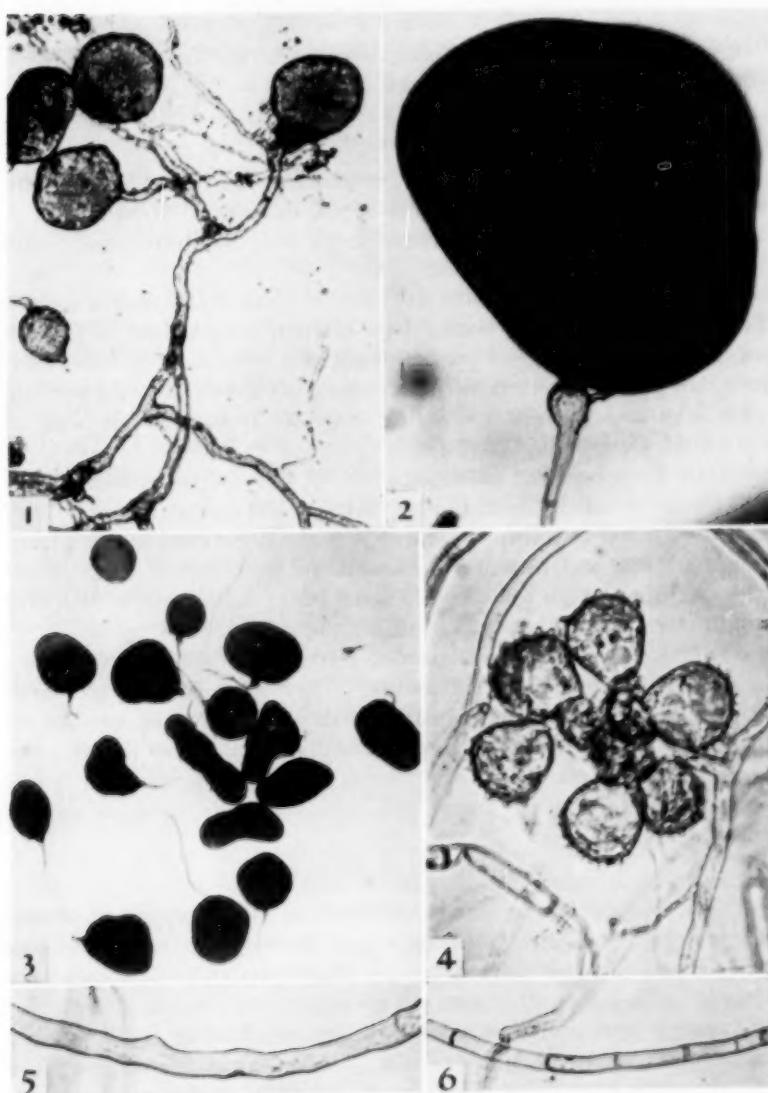
FIGS. 1–6. 1. Type A spores from field soil collected near red clover roots, × 138. 2. Type B spore from field soil collected near red clover roots, × 138. 3. Type B spores showing range in size and shape, × 20. 4. Type C spores on a hypha produced from a type B spore, × 450. 5. Coenocytic hypha produced by a B spore, × 450. 6. Septate hypha produced by a B spore, × 450.

produces a coil from which many smaller hyphae arise. The smaller hyphae branch again and tightly coil around each other. These branches eventually terminate in C spores.

DISTRIBUTION OF SPORES

Although complete records on the occurrence of type A spores were not made, they were abundant in most soil examined. Even though A and B spores were closely associated in the soil, they were never found attached to the same mycelium.

Type B spores fall within the size range of cysts of *Heterodera* species. Triffitt (13) reported a similar type of spore as common in English soils, and stated that it had been confused with cysts of the golden nematode. In personal conversations, nematologists have reported observing what may have been B spores, or a similar type, found in soils in California, Oregon, and Hawaii.

Type B spores were found in 16 of 19 soil samples collected from widely scattered red clover fields in northern and central Illinois. They also were found in soil from a three-year-old strawberry bed in Champaign, Illinois, and they were abundant in the Morrow Plots where corn had been grown continuously since 1876. Collections of B spores from the various sources appeared indistinguishable.

Type C spores have been found on mycelium growing from B spores in artificial inoculations or in culture. They have not been recovered from soil. Because of their small size they would not be retained on the sieves unless attached to considerable mycelium. Even though some C spores were retained by the sieves, a higher magnification would be required to observe them than was ordinarily used in routine examinations.

GROWTH IN CULTURE

Type B spores were surface sterilized by the following treatment. They were first washed by transferring them with a needle through two changes of sterile charcoal-treated distilled water. The spores were then placed in a dropper of water in a slightly tilted Petri dish. In order to insure a complete sterilization, those spores floating on the surface of the water were sunk with a needle. A dropper of 0.5% sodium hypochlorite made from commercial Clorox was added to the water containing the spores. This solution was removed with a dropper as rapidly as possible and replaced with sterile water. Spores were not in contact with the hypochlorite solution for more than 20 seconds. The spores were washed as rapidly as possible through at least three changes of

sterile charcoal-treated distilled water by adding and removing water with a dropper. They were then transferred to an agar medium and incubated at 20° C. Many spores were surface sterilized effectively by this treatment and in some trials as many as 40% germinated to form pure cultures. A few times all spores failed to germinate and appeared to have been killed by the treatment.

In two or three days, spores produced one or more germ tubes from the base of the spore near the bulbous swelling. The appearance of hyphae was similar to that obtained near artificially inoculated plant roots (FIGS. 5, 6). At first they were non-septate, irregular, up to 11 μ in diameter. As they grew away from the spore, they became smaller and more regular. Finally the hyphae became smooth, regularly septate, as small as 3 μ in diameter, branching at right angles, somewhat resembling *Rhizoctonia*. The fungus was grown on hemp seed agar (2 hemp seeds in 10 cc of water agar), potato dextrose agar, corn meal agar, malt agar, in water culture with bits of hemp seed, and in water containing non-sterile soil organic matter. On agar, growth was sparse and limited and hyphae did not exceed 22 mm in length. Somewhat better growth was obtained in water culture with bits of hemp seed or non-sterile soil organic matter; under these conditions a few type C spores were produced.

Hyphae and spores stain yellow with zinc chlor-iodide indicating the absence of cellulose. This confirms the experience of Galland.

INOCULATION WITH B SPORES

Type B spores extracted from soil have been used to inoculate plants of several different genera. Since it is desirable to use relatively small numbers of spores in inoculating, a method was devised for directing the roots into close contact with the spores. Seedlings were grown in funnels containing quartz sand with the spores placed at the top of the neck (FIG. 7). Small cotton plugs were placed in the end of the funnel and the neck was filled with sterile quartz sand. B spores that had been carefully washed in two changes of sterile distilled water, or surface sterilized, were transferred to the surface of the sand. The funnel was then filled with sand and surface-sterilized seed planted near the top. Small-seeded species were planted in 1½-inch funnels infested with 20 or 25 spores, and 4-inch funnels containing 50 spores were used for corn. Glass funnels were used in the first experiments. However, root growth plugged the neck of the funnel, thus slowing down the movement of water into the funnel as well as making removal of the roots difficult.
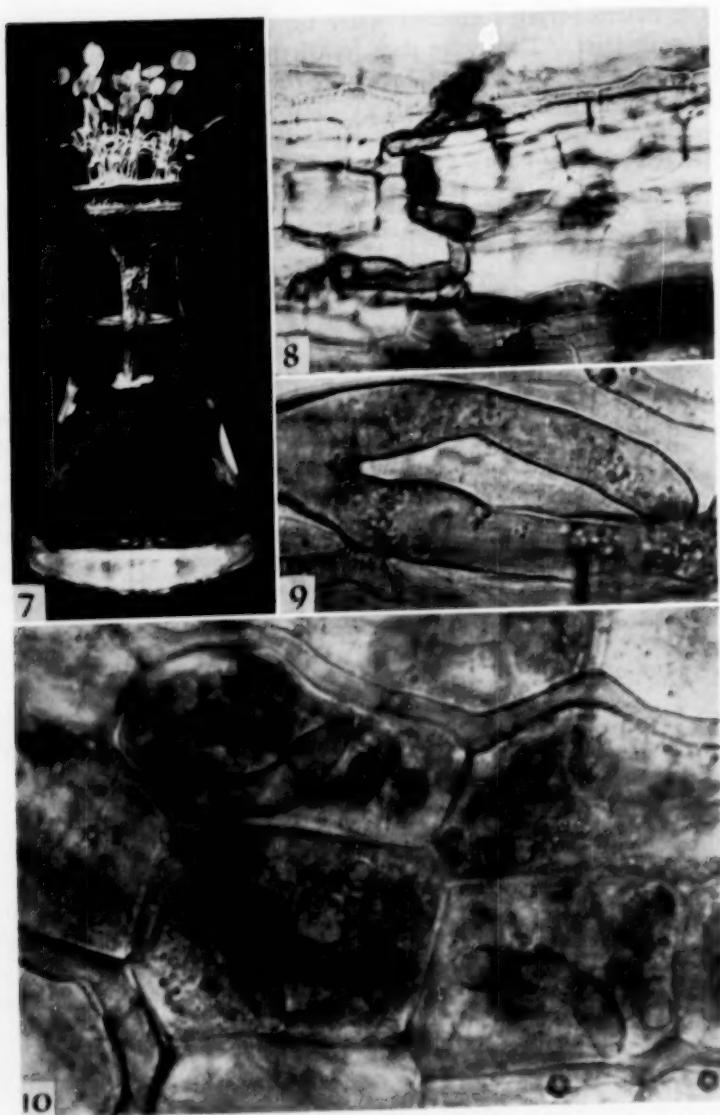
FIGS. 7–10.   Infection of red clover roots inoculated with B spores from red clover.   7. Inoculation method.   Two-week-old red clover seedlings growing in an aluminum foil funnel infested with B spores.   8. Penetration of the root.   Dark stained cells in the inner cortex contain arbuscles, × 450.   9. Continuous loop formed by penetrating hypha in the outer cortex, × 1030.   10. Intercellular hyphae and arbuscules in the inner cortex, × 1030.

Funnels made by forming a sheet of aluminum foil around a glass funnel were found to be more satisfactory. The neck of such a funnel expands, preventing plugging, as roots grow. Funnels must be watered from above until the seedlings emerge. Funnels placed with the neck in a flask of water at the start of an experiment became waterlogged and seed failed to germinate. After emergence, the funnels were placed on an Erlenmeyer flask of water. After two weeks a 1N Hoagland's solution was placed in the flasks for one day. Nutrient solutions cannot be allowed to remain in the flask for longer periods because evaporation concentrates the salts at the top of the funnel, resulting in poor growth. One watering with a nutrient solution gave satisfactory growth for a month.

After one month plants were removed from the funnels and the roots examined. Roots from plants grown in check funnels appeared free of fungi. In the infested funnels, B spores germinated and produced considerable hyphal growth (FIGS. 5, 6) around the roots. The hyphae varied from coarse, irregular, and non-septate to fine, smooth, and regularly septate. If penetration had occurred, C spores were present on this mycelium.

Hyphae grew along the surface of the roots, produced appressoria-like structures, and penetrated the epidermal cells directly. Hyphae grew intracellularly (FIG. 8) as they passed through the outer cortex and were constricted as they passed through cell walls. Penetrating hyphae were often loosely coiled, and continuous loops (FIG. 9) sometimes formed. Growth within the roots was almost completely coenocytic. Following penetration of the root, growth was mainly intercellular and confined to the inner cortex. Hyphae formed characteristic pointed projections between the rounded corners of cortical cells and arbuscules were produced in abundance (FIG. 10).

The arbuscule (FIG. 11) consists of coarse intracellular hyphae from which grow many small dichotomously branched tubes. The tubes are about $1\,\mu$ in diameter and difficult to observe. They are short-lived and appear to disintegrate shortly after they form. As the tubes break down, granular material[2] appears in the cell. This material becomes dense and may almost completely obscure the hyphal structure. As the arbuscule becomes older the granular material disappears. The coarse hyphae may also break down but often persist in the cell after the

[2] This material is usually referred to in the literature as "sporangioles." Since this is not a process of sporulation the use of this term as applied to the granular material should probably be discontinued.
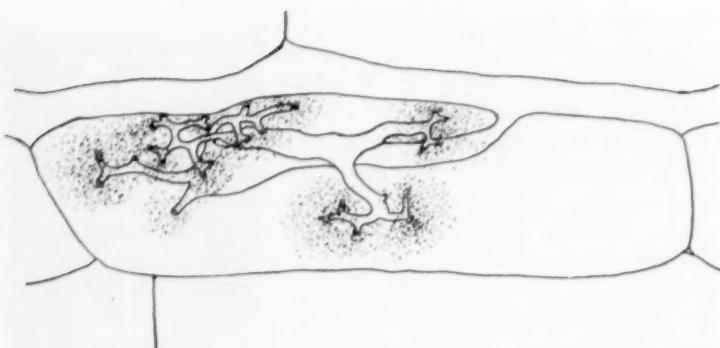
FIG. 11. Arbuscle in a cortical root cell of a strawberry seedling inoculated with B spores obtained from red clover showing the formation of granular material from the break down of the small tubes, × 984.

granular material has disappeared. Figures 12–16 show arbuscules in a strawberry root after most of the tubes and granular material have disappeared.

In one experiment the development of the fungus within red clover roots was observed over a longer period of time. Six funnels, each infested with 25 B spores, were planted to red clover. Six control funnels also were seeded with red clover. Water in which the spores had been washed was poured over the sand in the control funnels. After 17 days the aluminum foil funnels were removed, with as little disturbance of the sand as possible, and the plants transferred to pots of steam-sterilized soil. Over a period of three months roots were removed from the pots, cleared and stained, and examined microscopically. Roots from control plants remained free from the fungus. The fungus developed extensively within the roots of inoculated plants and type C spores were produced in abundance on external mycelium. Internal or external vesicles (type A spores) have never been produced on plants inoculated

TABLE I

CROSS INOCULATION WITH B SPORES FROM TWO SOURCES

| | Source of spores | |
Species tested	Red clover	Corn
Red clover	+	+
Corn	+	⎯
Strawberry	+	⎯ *
Sweet clover	+	⎯
Oats	⎯	⎯

* Negative results in this case may have been due to poor germination of spores.
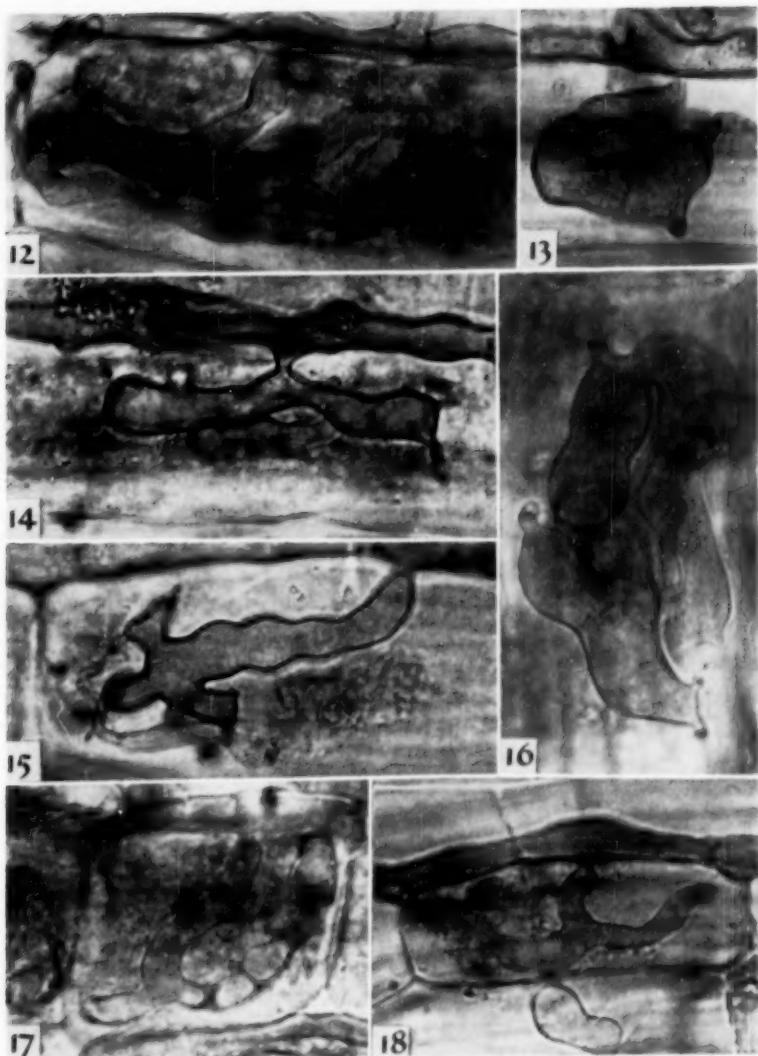
FIGS. 12–16. Arbuscles formed in roots of strawberry seedlings inoculated with B spores from red clover showing the appearance of the coarse hyphal structure after most of the fine tubes and granular material have disappeared, × 1030. FIGS. 17–18. Similar arbuscles from B and A spores, × 1030. 17. Arbuscle in a red clover root inoculated with B spores. 18. A similar type of arbuscle in a red clover root inoculated with A spores.

with B spores. A few immature B spores were found at the end of three months.

By inoculating with spores obtained from known hosts, it is possible to conduct a host-range test. Results obtained from inoculation of various crops with spores from clover and corn are given in TABLE I. Hyphae from B spores obtained from corn invaded red clover roots and produced arbuscules. Hyphae from B spores from red clover invaded corn roots, producing typical arbuscules.

Type B spores from clover also infected roots of strawberry and sweet clover, *Melilotus alba* Desr. Typical C spores were produced in every case where invasion of roots occurred. The results obtained from these experiments indicate a wide host range.

INOCULATION WITH TYPE A SPORES

The funnel technique was used to inoculate red clover plants with type A spores. In four experiments using type A spores, limited infection was obtained only in three roots. Penetrating hyphae, intercellular hyphae in the inner cortex, and arbuscules appeared very similar to, if not identical with, those produced in roots inoculated with B spores (FIGS. 17, 18). One group of typical C spores was formed, but no A or B spores were produced in these limited experiments.

DISCUSSION

Type B spores are among the largest produced by any fungus. The chlamydospores of some members of the Endogonaceae approach their size. Thaxter (12), in his revision of the Endogonaceae, reports that the chlamydospores of *Glaziella vesiculosa* Berk. measure 200×200–$415 \times 380 \mu$. However, the spores of all members of the Endogonaceae, as presently constituted, are borne in compact sporocarps. If B spores represent a sexual stage, then the resemblance of this fungus to members of the Endogonaceae must be superficial. The general appearance of the B spore and attached hyphae suggests that it may be a type of oospore. However, the absence of cellulose in hyphae and spore walls argues against this possibility. A study of its development may help to determine its nature.

Type C spores are slightly suggestive of the vesicles produced by *Endogone vesiculifera* Thaxter. The vesicles of *E. vesiculifera* differ in that they are larger, more clavate, thinner walled, nearly empty, and occur in groups with spherical thick walled chlamydospores. It should be noted, however, that Peyronel (11) claimed to have found connecting

hyphae between fruiting bodies of *Endogone vesiculifera* and an endo-
phyte of the phycomycetoid type in the roots of certain alpine plants.

The relation of type A spores to B spores is uncertain.   The hyphae
produced from A and B spores are similar in appearance.   The two
spores are closely associated in the soil and the attached hyphae are often
entangled.   However, they never have been found attached to the same
hypha.   Failure to obtain type A spores in or around roots inoculated
with B spores could be due to unfavorable environmental conditions, or
type A spores may be produced by another species.   If two species are
involved, the similarity of the infections obtained from A and B spores
would indicate that they are closely related.

The failure of pure cultures obtained from B spores to make con-
tinued growth suggests that this fungus may be an obligate parasite that
draws upon the stored food in the spore to grow considerable distances
through the soil.   However, this is uncertain and more attempts to
maintain cultures should be made.

SUMMARY

A large, yellow, one-celled spore 291–812 μ in diameter was found
to be common in Illinois soils.   Inoculation studies showed that it causes
a mycorrhizal infection of the phycomycete type.   The appearance of
the spore and attached hyphae suggest that it is a sexual stage.   These
spores have been separated in quantity from soil by wet sieving and
decanting.   They have been surface sterilized and pure cultures grown
from them.   Spores germinated by producing germ tubes.   Hyphae
varied from coarse, irregular and coenocytic to fine, smooth and regu-
larly septate.   It was grown on various types of agar media, but only
a limited growth, not exceeding 22 mm in length, was obtained.   Some-
what better growth was obtained in water culture with non-sterile soil
organic matter or bits of hemp seed.

When artificial inoculations were made, hyphae from these spores
penetrated plant roots.   Hyphae were intracellular as they passed
through the outer cortex and when they reached the inner cortex they
became intercellular as they grew through the root.   Arbuscles were
produced in cortical cells just outside the endodermis.   Hyphae within
the roots were non-septate.   After penetration, tight bunches of echinu-
late spores formed on the external mycelium.   The internal and external
vesicles often associated with this type of mycorrhiza in the field have
not formed in artificial inoculations.

Cross inoculation experiments utilizing spores from known sources

indicate a wide host range. What appeared to be the same strain infected roots of red clover, corn, strawberry, and sweet clover.

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THE MORPHOLOGY AND NUTRITION OF
A NEW SPECIES OF SIROLPIDIUM

Helen S. Vishniac

(WITH 8 FIGURES) (2 cuts)

Clam and oyster larvae grown at the U. S. Fish and Wildlife Service Laboratory at Milford, Conn. have suffered from time to time from outbreaks of a fungus parasite tentatively identified as a *Sirolpidium* (3). This fungus has now been isolated and grown in pure culture, thus obviating the difficulties, well described by Sparrow (9), of attempts to study marine phycomycetes in their natural hosts. Neither the morphology of this fungus in pure culture, nor its host range, permits assignment to any previously described species. It is therefore described as a new species. The species here erected is based primarily upon host range, since the distinguishing morphological features appear primarily in flourishing older cultures. This is the first marine phycomycete to be described in pure culture (though S. W. Watson [1] isolated *Thraustochytrium proliferum* in 1951). Studies of previously described species in pure culture will undoubtedly extend our ideas of their capacity for development.

ISOLATION

While it should be possible to isolate holocarpic marine phycomycetes as Couch (2), and later others, have isolated some of the non-filamentous freshwater and terrestrial Phycomycetes, by careful washing and the use of very dilute inocula, it is much quicker—and easier in view of the perishability of the material, the small size of the zoospores, lack of foreknowledge of suitable media and conditions of growth, and the possibility of inhibition by competing microorganisms—to use antibiotics as adjuvants to isolation. The use of penicillin and streptomycin to sterilize unwanted bacteria has become widespread; organisms otherwise difficult to isolate, such as myxomycete plasmodia (8) and marine plankton (12), among others, have yielded readily to this technique. Although it is known that streptomycin can produce heritable changes in some algae and higher plants (7), neither antibiotic has any apparent effect on fungi.

To isolate the clam parasite, plates of a nutrient agar (sea water 90%,

[1] Personal communication.
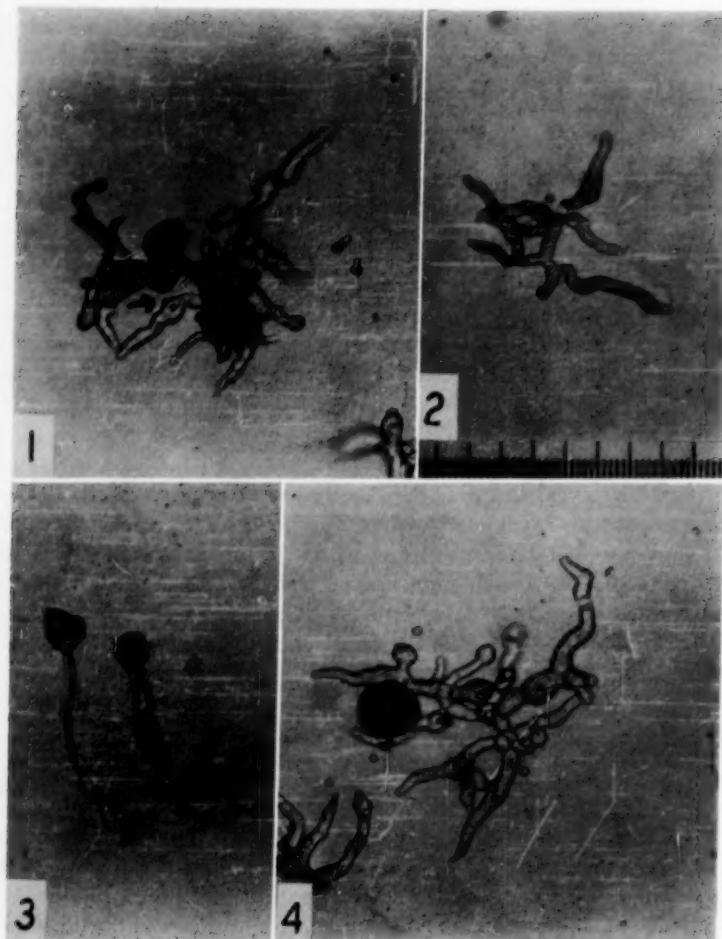
FIGS. 1–4. *Sirolpidium zoophthorum* grown on agar, *ca.* × 92 (scale between figs. 2 and 4; 1 division = 10 μ). 1. Germinating cystospores at right. Thallus with two mature sporangia. Arrow indicates discharge tube of hidden sporangium. 2. Young branching thallus. Apparent hiatus is large vacuole. Septa and other vacuoles present but obscured. 3. Old olpidioid thalli showing branching "rhizoidal extensions." 4. Vacuolate thalli with "resistant cell."

gelatin hydrolysate 0.1%, water soluble vitamins after Vishniac and Watson (**14**)) were poured, dried overnight at 37°, and spread with 0.2 ml aliquots of an infected larval culture together with 200 units of crystalline penicillin G and 0.5 mg of streptomycin. After two to ten days

incubation at 20° the spread plates were found to contain colonies of yeasts, *Sporocytophaga* (?), three species of holocarpic marine phycomycetes and, growing out from the clam larvae, the desired parasite. Infected larvae sufficiently clear of other fungi were removed from the plate with a fine needle and placed in drops of sterile 90% sea water on fresh nutrient agar plates (as above with the addition of filter sterilized glucose 0.05% and yeast extract 5.0 mg %). Infected larvae were also placed in small tubes containing 1.0 ml of 90% sea water. Subsequent tests of this sea water did not indicate the presence of zoospores, nor were zoospores visible on microscopic examination of these tubes. The thalli placed in drops of sea water began to produce zoospores in about 5 hours. These zoospores, when transferred to another plate of the same nutrient agar, germinated and grew in the absence of clam larvae, and gave rise to an abundance of material for subcultures and further study.

MORPHOLOGY

GERMINATION AND GROWTH TO YOUNG MATURE THALLUS: When a group of zoospores is transferred to a freshly poured plate of a suitable nutrient agar and incubated at 20°, they soon cease to swarm, settling as more or less spherical masses upon the agar. (The nutrient agar used for these studies contained sea water 90%, gelatin hydrolysate 0.1%, separately sterilized glucose 0.05%, vitamins as before, and agar 1.5%.) The cyst thus formed enlarges isodiametrically until it has reached a diameter of approximately $7-10 \mu$. At this point elongation of the thallus commences, forming a regular tube, also approximately $7-10 \mu$ in diameter but frequently slightly narrower than the cyst (FIG. 1). Rarely the cyst sends out two such tubes so that the initial hypha is branched in appearance. In thalli which have reached a length of about 40μ a large spherical vacuole is visible. These vacuoles, set in foamy cytoplasm and approaching or equalling in diameter the diameter of the thallus, are among the most conspicuous features of this fungus. In thalli of $10-14 \mu$ diameter, the vacuoles are $9-13 \mu$ in diameter (FIGS. 2, 4).

With little further elongation of the thallus, septa appear, delimiting cells $10-14 \mu$ in diameter and $42-98 \mu$ in length. The thallus then begins to branch, usually by the continued growth of a non-terminal cell to one side, past the septum between it and the next younger cell, much as secondary sporangia are formed in *Achlya*. Less frequently branches originate from the middle portion of the cell. The branches thus formed may or may not be cut off later by a septum at the point of union with the parent cell. When, after achlyoid branching of a non-terminal cell, the next younger cell sends out a branch directed posteriorly at the same

septum, an X-shaped mycelium develops which might be mistaken for hyphal fusion if the stages of its development had not been observed. A typical young branching mycelium measures $138\,\mu$ in length with a $61\,\mu$ branch continuous with the first of the two cells, and is about $15\,\mu$ wide, slightly irregular in width and in shape.

By the third or fourth day of growth the thallus is a much branched and dense structure, continuing to grow in length at many growing points and in thickness of the individual cells. In the interior of the mycelium the cells become very irregular in shape, up to $46\,\mu$ in diameter, while some of the terminal cells have swollen into obovoidal structures, 50–$80\,\mu$ at their largest diameter, filled with dense cytoplasm. Pre-terminal cells are 15–$25\,\mu$ in diameter.

SPORULATION: If a drop of sterile 90% sea water is placed on such a thallus one or more of the swollen terminal cells act as sporangia, each producing a single (rarely 2) discharge tube of uniform thickness ($5\,\mu$) varying in length from 15 to $142\,\mu$, fairly straight except for the longer forms. Zoospores are cleaved out within the sporangium and, after swarming within the sporangium, swim at random through the discharge tube. The tip of the discharge tube appears simply to deliquesce. The discharge of zoospores commences about four hours after the application of the water drop to a suitable thallus. Three-day cultures contain comparatively few mature sporangia.

The zoospores are about $2 \times 5\,\mu$, more or less pear-shaped in motion, at rest varying from ovoid to fusiform to irregularly triangular. Under the high power of the light microscope they not infrequently appear to have a single stout flagellum (cf. de Bruyne's *Olpidium Bryopsidis*, 4). The motion of the zoospores is, however, the typical tumbling progress of the heterokont spore. Electron micrographs confirmed that the spores were heterokont (FIG. 8). Swarming has been observed to continue for nearly 24 hours, though it is usually terminated earlier by the drying of the drop of water used to induce sporulation. Reflooding does not induce further swarming, once the spores have encysted. From this I have assumed that the zoospores are monoplanetic.

DEVELOPMENT OF RHIZOIDAL EXTENSIONS: After induction of sporulation, that part of the thallus which has not sporulated continues to grow more slowly, the individual cells bulging irregularly to 56–$82\,\mu$, with vacuoles reaching $25\,\mu$ in diameter. As the medium becomes exhausted the cells send out one to several extensions into the agar, on the surface of the agar, and into the air if the atmosphere is sufficiently humid (FIG. 3). These extensions tend to taper from base to tip from as much as 12–$16\,\mu$ to 4–$5\,\mu$, though the diameter through most of the length is

5–6 μ. They thus resemble discharge tubes in diameter, except for the taper. They are unlike discharge tubes in length (178–642 μ), frequent branching and rebranching, and in that several usually occur on one cell. When such a cell is induced to sporulate, it does not discharge through such an extension, but produces a new discharge tube through which the spores produced within the cell (sometimes including the thicker basal portion of the extensions) swim. Transfer to fresh medium does not result in the production of new thalli from these extensions, which can not, therefore, be considered hyphae. If one considers that these extensions may act as absorbers of nutrients at a distance from the thallus, though they do not have the anchoring function of rhizoids, it seems justified to refer to them as rhizoidal extensions. The possession of such extensions, and the fact that although apparently any cell of an older thallus may function as a sporangium, the entire thallus never does so on flooded plates, make it difficult to conclude that this fungus is strictly holocarpic.

RESISTANT CELLS : Occasionally there are produced, as side branches on the thalli, light golden-brown ovoidal cells, approximately 40 × 45 μ to 80 × 90 μ, with dense cytoplasm and relatively thick walls (FIG. 4). Such structures also appear in thalli grown in semi-solid (0.1% agar) media, and may be produced directly from zoospores under the conditions which give rise to olpidioid thalli. Their structure suggests that they may be resistant cells. Whether they are, and if so, what they resist, are problems which must await knowledge of the conditions which allow regular and abundant production of these cells. Resistant cells germinate by the production of hyphae (ca. 10–15 μ in diameter) giving rise to the usual thallus.

"Gemmae," that is, cells with thick walls and dense, non-vacuolate protoplast, but of the various sizes and shapes and lack of color of the vegetative cells, are found in thalli grown on semi-solid media (FIG. 7). These cells do not withstand desiccation but are more resistant to plasmolysis than the vegetative cells. Exposure to sea water concentrated fourfold rapidly plasmolysed vegetative cells, but only slowly plasmolyzed the gemmae. After three hours in such concentrated sea water, gemmae, but not vegetative cells, were capable of producing a normal thallus when plated on fresh media. Both types of cells survive dilute sea water (10%) to some extent, though after three hours many disintegrated cells of both types can be seen.

OLPIDIOID THALLI : Zoospores or cysts allowed to germinate upon dry plates (i.e. plates not used for several days after pouring), on plates exhausted of nutrient by the parent thalli, or on freshly poured plates at

30°, do not produce the thallus described above. Most continue to grow more or less isodiametrically to produce a non-vacuolate, spherical to ovoid, "olpidioid" thallus. Many produce thalli consisting of one or two hyphae soon terminated by bulbous swellings. An occasional more normal, but usually non-vacuolate, thallus appears. Mere crowding on a moist, freshly poured plate at 20° does not produce olpidioid thalli. Olpidioid thalli from a seven-day culture measured 25 to 31 μ (spherical thalli) and 31 × 33 to 46 × 56 μ (ovoid thalli). Smaller and larger thalli from younger and older cultures are also capable of acting as sporangia.

PRODUCTION OF SPORANGIA BY "FRAGMENTATION": Old cultures, or those on dry plates, or grown at 30°, exhibit another peculiarity: the cell contents pull away from the cell wall (especially at the ends), rounding into one or more protoplasmic masses (FIGS. 5, 6). These bodies secrete new cell walls, which can be clearly seen within the old cell outlines after sporulation has been induced, emptying the new cell. These sporangia, formed by fragmentation of the protoplast within the cell, are strongly reminiscent of Sparrow's figure (9, Pl. 2, fig. G) of *Sirolpidium Bryopsidis*.

Sirolpidium is, in fact, separated from *Pontisma* (the other monospecific genus of the Sirolpidiaceae) by the characteristic fragmentation of its lagenidioid thalli to form discrete olpidioid sporangia. Although Sparrow (9) remarks, apropos of the figure cited above, that "often in such chains of cells the wall of the original vegetative body can be detected between the segments," both Sparrow (9, Pl. 2, fig. E; 10, Pl. I, fig. 4) and Petersen (6, Fig. IX–2, 3) have figured olpidioid sporangia formed by fragmentation of the thallus (i.e. separation of entire cells) rather than of the protoplast of *Sirolpidium Bryopsidis*. Fragmentation of the thallus of the present fungus, when it is grown on plates, has not been observed.

Sparrow (10) also observed that "if infected *Bryopsis* were put in close contact with air, such as allowing water to drop over them rather than submerge them entirely, the fungus underwent a remarkable transformation in body structure. Instead of the formation of typical olpidioid thalli, there were formed definitely filamentous, ramifying hyphae." This observation of Sparrow's suggested that in *Sirolpidium*, as Bail (1) and Pasteur (5) found for *Mucor* and other fungi, fragmentation of the thallus may occur as a result of exposure to partially anaerobic conditions. This is at least partially true of the present fungus. When grown in approximately 1 cm layers of semisolid (0.1% agar) or 5 ml portions of liquid medium in tubes, it segments into fragments of 2 to 7 cells, frequently including one or more branches, measuring 148 μ (two cells) to 303 μ
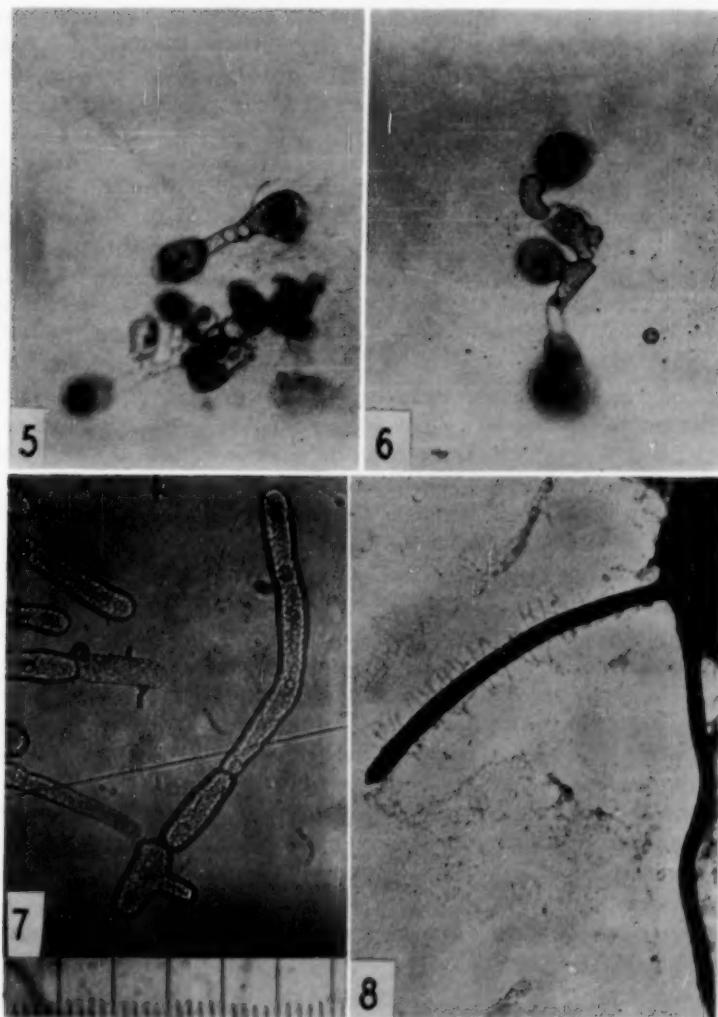
FIGS. 5–8. *Sirolpidium zoophthorum.* 5, 6. Old thalli showing fragmentation of the protoplast. 7. Grown in liquid culture, *ca.* × 156 (scale below; 1 division = 10 μ). Three-celled thallus fragment. 8. Zoospore, *ca.* × 14,200. Flagella broken in preparation, but clearly showing "tinsels" on one, not on the other.

(four cells) in length (excluding branches), and 10 to 15 μ in diameter
(FIG. 7). Separation of individual cells has not been observed, but
presumably could occur under more nearly anaerobic conditions such as
in the presence of oxygen-utilizing bacteria.

<div align="center">SYSTEMATIC POSITION</div>

The foregoing description of the morphology of the clam parasite
places it in the Sirolpidiaceae of the Lagenidiales of the Biflagellatae.
This marine family is characterized, according to Sparrow (11), by an
endobiotic, holocarpic thallus lacking in rhizoids, with few or no branches,
strongly vacuolate. Monoplanetic, biflagellate zoospores are formed
within the sporangium and emerge through a single discharge tube, or
occasionally more than one. Sexual and resting stages unknown. To
this should be added that, as Petersen (6) emphasized, both of the genera
of this family produce both olpidioid and tubular, septate (lagenidioid)
sporangia. The difficulties of placing the clam parasite in the Sirolpidi-
aceae are two: first it is dubiously holocarpic; secondly, it produces a
vigorous, well-branched thallus. The extensive development of the
thallus is undoubtedly due to the conditions under which it has been
cultivated; less extensive thalli can be produced at will by modifying the
conditions of culture as described. As to the first difficulty, the produc-
tion of rhizoidal extensions is probably not peculiar to the present fungus.
Sparrow's (10) "hyphae" of *Sirolpidium Bryopsidis* (Pl. I, fig. 7) and
(9) "long, branched discharge tube" of *Pontisma lagenidioides* (pl. 3,
fig. E) seem to have the appearance and dimensions of rhizoidal exten-
sions rather than hyphae (which are usually thicker) or discharge tubes
(which, in the clam parasite at least, do not branch).

Since the definitive character of *Sirolpidium*, as opposed to *Pontisma*,
is fragmentation of the thallus, which in many fungi is entirely dependent
upon environmental conditions, it is doubtful that *Pontisma* is a valid
genus. For this reason, although *Pontisma* can hardly be disestablished
until pure culture studies have been made of both *P. lagenidioides* and
S. Bryopsidis, the clam parasite is here placed in *Sirolpidium*, the prior
genus. In morphology it differs from *Sirolpidium Bryopsidis* (as de-
scribed on natural hosts) in the development of a highly branched, exten-
sive, non-fragmenting thallus under favorable conditions, from *Pontisma
lagenidioides* (as described on natural hosts) in protoplasmic and cellular
fragmentation under unfavorable conditions. The dimensions of the
thallus, discharge tubes and zoospores are essentially similar for all three
fungi. *Sirolpidium Bryopsidis* has been reported only on marine

Chlorophyta (de Bruyne, **4**; Petersen, **6**; Sparrow, **10**, on *Bryopsis plumosa;* Sparrow, **10**, on *Cladophora* sp.); *Pontisma lagenidioides* on four species of the rhodophyte *Ceramium* (Petersen, **6**; Sparrow, **9**, **10**). The present fungus parasitizes larvae of *Venus mortoni, Crassostrea virginica,* and *V. mortoni* × *V. mercenaria* hybrids (**3**). This great difference in natural host seems at present sufficient reason to place the present fungus in a new species:

Sirolpidium zoophthorum sp. nov.

Thallus ramosus septis praeditus; cellulae quaeque vacuolo magno sphaerico pra·ditae, iuvenes 10–15 μ diam., ad maturitatem usque ad 82 μ diam. irregulariter crescentes; seniles rhizoideis ramosis praeditae. Sporangia primum apice lata, deinde cellula quaevis sporangium fiere potens. Zoospora monoplanetica, biflagellata, ca. 2 × 5 μ, in sporangiis formata et per tubulum 5 × 15–142 μ singulatim natantes. Thalli sub cultura infelici unam cellulam, ovoideam vel sphaericam, sistentes, sporangia fieri potentes. Spora resistens ca. 40 × 45 μ ad 80 × 90 μ, fulva, membrana crassa praedita. Gemmae quoque formatae.

Parasiticum in larvis *Veneris* et *Crassostreae,* Milford, Conn., U. S. A.

PHYSIOLOGY AND NUTRITION

Sirolpidium zoophthorum makes good growth at 20, 25, and 30°, but does not grow at 36° and survives poorly under refrigeration. Stock cultures are kept in liquid media at room temperature and transferred once a week. The stock medium consists of marine mineral base (Vishniac, **13**); NaH·glutamate, 0.05%; agar (Difco Bacto), 0.1%; thiamine·HCl, 20 μg %; and glucose (aseptically added after separate sterilization), 0.05%, at pH 7.5. In this medium *S. zoophthorum* grows at initial pH from at least 7.5 to 6.7, but not at initial pH below 6.5. Growth tends to bring the medium to neutrality.

For nutritional experiments the stock medium, with the omission of the constituent in question, was used, distributed 10 ml in 25 ml glass-capped Erlenmeyer flasks. After sterilization and inoculation with one drop of thallus fragments from a mature liquid culture, these flasks were sealed between pyrex kitchen trays with Scotch tape and incubated at 25°. Growth was estimated by visual inspection. A seven-day culture grown on stock medium under these conditions has a dry weight of 5.4 to 9.0 mg, depending upon the method of harvest. The fineness of the thallus and the presence of small amounts of agar (required for support in stationary culture) in the medium made the determination of dry weights too cumbersome for routine use. The smaller figure was obtained by centrifuging the culture and removing as much as possible of the fungal layer alone to a dried, weighed filter paper disc on which it

TABLE I

GROWTH OF SIROLPIDIUM ZOOPHTHORUM IN VARYING CONCENTRATIONS OF NaCl
AFTER 6 DAYS AT 25°

NaCl	0	1.0%	1.5%	2.0%	2.5%	3.0%	3.5%	4.0%
Growth	0	+*/++	++/+++	+++/+++	+++/+++	+/+*	?/+	0/?

0 indicates no growth; ? uncertainty as to whether growth occurred or not;
+, +*, ++, +++ increasing amounts of growth from slight to very good.

was dried. The higher figure was obtained by diluting with water and
tediously filtering the culture under suction, with washing, onto a dried,
weighed filter paper disc on which it was dried. The true dry weight
is probably somewhat higher when the first method is used, somewhat
lower when the second is used.

INORGANIC REQUIREMENTS: *Sirolpidium zoophthorum* is obligately
marine—or estuarine—failing to grow if NaCl is omitted from the mineral
base. Growth occurs if 1.0 to 3.5% NaCl is present, with a broad max-
imum at 2.0 and 2.5% (see TABLE I). KCl may be omitted from the
medium without significantly affecting growth. Further investigation of
inorganic requirements was not made because this fungus, which is un-
suitable for quantitative work, grows as well or better in the synthetic

TABLE II

GROWTH OF SIROLPIDIUM ZOOPHTHORUM AS AFFECTED BY THE PRESENCE OF
ORGANIC AND AMINO ACIDS RELATED TO THE KREBS CYCLE, AFTER INCUBATION FOR
7 DAYS AT 25°

Addition		initial pH 7.58		initial pH 6.73	
			+NaH.glutamate 50 mg %		+NaH.glutamate 50 mg %
none		±	++++	+	++++
NaH. glutamate	5 mg %	++	++++	+*	++++
	10 mg %	++	++++	++	++++
	20 mg %	++	++++	+++	++++
	50 mg %	++++	++++	++++	++++
(K)L-aspartate	20 mg %	+	++++	++	++++
	50 mg %	0	0	±	+++
Na₃ citrate.5½H₂O					
	20 mg %	0	+++	±	+++
	50 mg %	0	+++	+	++++
Na₂ succinate.6H₂O					
	20 mg %	0	+++	+	+++
	50 mg %	0	+++	0	+++
(Na) fumarate	20 mg %	±	++++	0	++++
	50 mg %	±	+++	±	+++

0 indicates no growth; ± barely visible growth; +, +*, ++, +++, ++++
increasing amounts of growth from slight to excellent.

stock medium as in sea water enriched with gelatin hydrolysate, vitamins and glucose. The NaCl requirement, and the absence of a high K⁺ requirement, are sufficient to indicate that, while obligately marine, this fungus is not exactingly stenohaline and is probably estuarine in habitat (see Vishniac, **13**).

ORGANIC REQUIREMENTS: In attempting to substitute known amino acids for the gelatin hydrolysate used in isolation the following groups of amino acids, each amino acid at 5.0 mg %, were supplied: a) DL-alanine, NaH·glutamate, (K) L-aspartate, and L-proline; b) L-arginine· HCl, glycine, and L-lysine; c) DL-isoleucine and DL-valine; d) DL-

TABLE III

AVAILABILITY OF VARIOUS CARBON SOURCES (0.05%) TO SIROLPIDIUM
ZOOPHTHORUM I CUBATED AT 25° FOR SEVEN DAYS

Carbon source		+glucose (0.05%)
none	+	+ + +
soluble starch	+ + +	+ + +
sucrose	+	+ +
cellobiose	±	+ + +
maltose	+	+ +
lactose	0	+ +
glucose	+ + +	+ + +
galactose	±	+ +
fructose	±	+ + +
arabinose	?	+ + +
xylose	?	+ + +
ribose	+	+ + +
ethanol	+	+ + +
butanol	±	+ + +
glycerol	±	+ +
mannitol	±	+ +
sorbitol	+	+ + +
Na.acetate.3H₂O	±	+ +
(Na) lactate	+	+ + +

0 indicates no growth; ? uncertainty as to whether growth occurred; ± barely visible growth; +, + +, + + + increasing amounts of growth from slight to very good.

tryptophan, DL-tyrosine, and DL-phenylalanine; e) L-leucine, DL-serine, and DL-threonine; f) L-histidine·2HCl; and g) DL-methionine. Groups b, c, and f were inhibitory, as evidenced by failure of the fungus to reach the slight growth of the control with the addition of 5 mg % of gelatin hydrolysate. Since none of the other groups gave consistently improved growth over the controls (without amino-N and with 5.0 mg % gelatin hydrolysate), it seemed probable that the requirement was of much greater magnitude than the usual amino acid requirement and was therefore for one of the amino acids closely related to the Krebs cycle. As is shown in TABLE II, NaH·glutamate at 50 mg % supports excellent growth, and (K) L-aspartate at 20 mg % supports good growth under

certain conditions, but the organic acids of the Krebs cycle, though not inhibitory, do not, even when the pH of the medium is lowered to favor absorption of the un-ionized acid.

Of 18 compounds (listed in TABLE III) supplied to *Sirolpidium zoophthorum* at 0.05% as sole carbon sources (in the presence of NaH·glutamate, which does not support continued growth as sole carbon source), only glucose and soluble starch supported growth greater than the barely visible growth in the control flask (TABLE III). All sugars were sterilized by filtration and added aseptically, as were ethanol and butanol, to sterilized medium.

On the third subculture in media from which vitamins have been omitted (except such as are present in the agar used), *S. zoophthorum* ceases to grow. The addition of thiamine·HCl, alone of the recognized water-soluble vitamins, restores growth to the level permitted by the mixture of water-soluble vitamins used in isolation.

SUMMARY

The morphological development in pure culture of *Sirolpidium zoophthorum*, a new species of marine phycomycete parasitizing clam and oyster larvae, is described. This highly aerobic fungus makes good growth at temperatures ranging from 20 to 30° in defined media containing appropriate inorganic salts, glucose or soluble starch, NaH·glutamate, thiamine, and small amounts of agar, provided that the initial pH is between 6.7 and 7.5.

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BIOSYNTHETIC POTENTIALITIES OF HIGHER FUNGI [1]

WILLIAM D. GRAY AND WILLIAM R. BUSHNELL [2]

(WITH 2 FIGURES)

In comparatively recent years considerable emphasis has been placed upon studies of the special physiology and industrial applications of fungi, thus alleviating in part the situation long existent in the field of mycology in which the emphasis was largely of a taxonomic and morphologic nature. Impetus to this broadening of viewpoint in mycology has been largely attributable to the various discoveries which have demonstrated that a variety of fungi are capable of synthesizing organic compounds of great value to man both industrially and medically.

Although studies of fungus metabolic activities had been variously reported earlier, the first and most extensive attempt to make a thorough investigation of the compounds synthesized by fungi was that of Raistrick, Birkinshaw and others whose basic papers were published in 1931. In their investigations, the metabolic products were separated into chemical categories of a general nature (*e.g.* neutral volatile compounds, acidic volatile compounds, etc.) and the total amount of carbon in each category was then carefully determined by a wet combustion method. Qualitative analyses of the compounds in the various general categories (especially the carbon unaccounted for category) subsequently led to the discovery of a great variety of organic compounds hitherto unknown as fungus metabolic products.

Unfortunately the work of Raistrick and his colleagues presented a somewhat restricted view of fungus metabolism, since their work was concerned largely with species of *Aspergillus*, *Penicillium* and other genera of the mold type of fungi. Thus, of nearly 250 fungi investigated, there were included only three species of Ascomycetes (other than *Penicillium* and *Aspergillus*) and two species of Basidiomycetes [*Ustilago Maydis* (DC.) Corda and *U. avenae* (Pers.) Jensen]. Although no species of so-called higher fungi were investigated, unfortunately there

has been some tendency to generalize regarding fungus metabolism on the basis of the researches on molds of Raistrick and his co-workers.

In view of the paucity of information concerning the metabolism of the fleshy Ascomycetes and Basidiomycetes, there was initiated in this laboratory a systematic study of the metabolism of such fungi, based upon the methods and techniques employed by Raistrick and his colleagues. Shortly after the initiation of this project Whitaker (**1951**) published an account of the carbon balances obtained from his work with 40 species of wood-rotting Basidiomycetes; the carbon balances which he presented were not as complete as those prepared by the English workers. The first work from this laboratory concerning the metabolism of Basidiomycetes was that of Allen (**1952**), who presented carbon balances for two species of *Coprinus,* two species of *Pleurotus,* two species of *Polyporus* and one species of *Hypholoma.*

In view of the possibility that certain of the higher fungi conceivably might synthesize no compounds other than carbon dioxide and the various assimilation products of which the mycelium is composed, it was felt that an attempt should be made to determine if an index to the biosynthetic potentialities of these fungi could be provided by methods other than the extensive ones employed in the preparation of complete carbon balances. The need for a simpler method of screening out the organisms judged most promising for more complete investigation seemed especially desirable in view of the report of Raistrick *et al.* (**1931**) that some of the organisms which they investigated did not synthesize appreciable amounts of any material other than carbon dioxide and mycelial compounds.

The present work reports the results obtained with a much less elaborate method for separating out those species of fungi which appear to convert a considerable portion of the glucose which they utilize to compounds other than carbon dioxide and permanent mycelium constituents.

MATERIALS AND METHODS

The fungi used in this investigation are from the stock culture collection maintained in this laboratory, and the numbers accompanying each species are the permanent stock culture numbers under which they are listed. All species were initially isolated by means of sporocarp tissue cultures, and all have been maintained in culture on 0.7 percent Difco Yeast Extract, 0.5 percent KH_2PO_4, 2.0 percent glucose agar. All of the test fungi were either Ascomycetes or Basidiomycetes. Of the ascomycetous species one (*Xylaria polymorpha*) was a pyrenomycete, the remainder were Discomycetes; this latter group contained 13 isolates

representing 6 genera and 11 species. Thirty-six different Basidiomycetes were used; of these, the family Agaricaceae was represented by the greatest number of species; however, there were also five polypores, four boletes, one trembling fungus, one bird's nest fungus and one puffball. The total number of basidiomycete species was 36; these were distributed in 24 genera. Test organisms and their stock culture members were as follows:

ASCOMYCETES

Bulgaria inquinans Fr.   #141
Bulgaria rufa Schw.   #42
Bulgaria rufa Schw.   #142
Leotia lubrica (Scop.) Pers.   #144
Morchella crassipes (Vent.) Pers.   #145
Morchella deliciosa Fr.   #147
Morchella esculenta (L.) Pers.   #148
Morchella hybrida (Sow.) Fr.   #119
Morchella hybrida (Sow.) Fr.   #149
Peziza badia Pers.   #151
Peziza venosa Pers.   #150
Sclerotinia tuberosa (Hedw.) Fuckel   #152
Verpa conica (Müll.) Schwartz   #190
Xylaria polymorpha (Fr.) Grev.   #154

BASIDIOMYCETES

Agaricus placomyces Peck   #174
Agaricus rodmani Peck   #123
Amanita caesaria Scop.   #173
Boletinus merulioides Schw.   #169
Boletus cyanescens Bull.   #165
Boletus indecisus Peck   #168
Boletus luridus Schaeffer   #166
Clitocybe illudens Schw.   #175
Clitocybe monodelpha Schw.   #137
Collybia radicata Relh.   #177
Collybia velutipes Curt.   #100
Coprinus atramentarius (Bull.) Fr.   #121
Coprinus comatus Fr.   #94
Coprinus radians (Desm.) Fr.   #91
Cortinarius armillatus (Alb. & Schw.) Fr.   #178

Cortinarius sp.   #179
Crepidotus latifolius Peck   #180
Crucibulum vulgare Tul.   #170
Favolus canadensis Kl.   #159
Ganoderma applanatum (Pers. ex Fr.) Pat.   #158
Hypholoma incertum Peck   #181
Hypholoma perplexum Peck   #97
Lepiota morgani Peck   #126
Lycoperdon gemmatum Batsch   #171
Mycena leaniana Berk.   #182
Omphalia chrysophylla Fr.   #183
Panus rudis Fr.   #120
Pholiota acericola Peck   #185
Pholiota praecox Pers.   #186
Pluteus cervinus Schaeff.   #184
Polyporus lucidus (Leys.) Fr.   #160
Polyporus pennsylvanicus Sumstine   #162
Polyporus tsugae (Murr.) Overh.   #138
Russula variata Bann.   #188
Tremella sp.   #155
Tricholoma albobrunneum (Pers. ex Fr.) Quél.   #189

Except for the maintenance of stock cultures, the medium used in all experiments was the chemically defined liquid medium described by Allen (**1952**), which is standard for metabolism experiments in this laboratory.   The initial pH of the medium (after autoclaving at 15 pounds pressure for 30 minutes) varied from 6.2 to 6.5.   Prior to inoculation, the initial pH was determined by means of a line-operated Beckman glass electrode, total glucose by the method of Stiles, Peterson and Fred (**1926**), and initial titratable acidity was determined by diluting a 5 ml sample to 100 ml with distilled water and titrating with 0.01 normal NaOH.

Medium was dispensed in 250 ml portions in 500 ml Erlenmeyer flasks which were then cotton-stoppered and autoclaved, and then each flask was inoculated with a small (*ca.* 5 mm diameter) bit of mycelium from an agar slant culture.   Each flask was then fitted with a sterile aeration set-up as shown in FIG. 1.   The aeration set-up was so designed that the air which was introduced into the flask did not bubble up through the medium but entered close to the liquid surface and served primarily to sweep out the gaseous contents of the culture flask through the series of tubes shown.   The air entering the culture flask was first

freed of carbon dioxide by passage through a 50 percent KOH solution followed by passage through a soda lime tower and was then freed of any possible contaminating organisms by passage through a sterile cotton filter. The exit tube from the culture flask passed into a series of four 25×200 mm test tubes, the first of which served as a trap, the second and third of which each contained 25 ml of 1.000 normal NaOH, and the fourth of which contained soda lime, which prevented the back diffusion of carbon dioxide from the atmosphere into the standard hydroxide solution. Air was passed slowly but continuously through the culture flask throughout the entire incubation period.

With the exception of *Xylaria polymorpha, Pholiota acericola* and *Boletinus merulioides* (Nos. *154, 185* and *169*) the different test fungi were used in only one experiment. Both *Bulgaria rufa* and *Morchella*
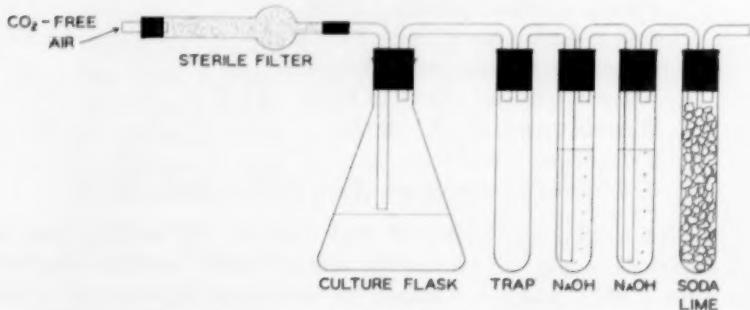
FIG. 1. Apparatus used for culturing fungi in metabolism studies.

hybrida are listed twice, but with these two species different isolates rather than duplicate experiments are involved.

All experiments were conducted at room temperature, which varied from 28 to 31° C. Cultures were incubated until good mycelial growth was obvious, the incubation time for the majority being between 40 and 60 days. At the end of the incubation period aeration was discontinued, the culture flasks and sodium hydroxide tubes were removed and the following final analyses were made:

Total volume.—Since all experiments were of relatively long duration, and a continuous stream of air had passed through the culture flasks throughout the incubation period, some reduction in total volume always occurred. Hence, upon removal from the system, the final volume of medium was measured and then made up to the original 250 ml with CO_2-free distilled water prior to taking samples for other analyses.

Final glucose concentration.—A reading was first taken with a Balling hydrometer in order to determine the approximate glucose concentration. Final glucose was then measured by means of the Stiles, Peterson and Fred method.

Final pH.—Final pH was measured with a line-operated Beckman glass electrode.

Final titratable acidity.—A 5 ml sample of the culture medium was diluted to 100 ml with distilled water and titrated with 0.01 normal NaOH. This measurement as well as pH measurement was made in order to gain some insight as to which species were strong acid formers.

Mycelium weight.—The mycelial pad in each flask was filtered out on a numbered, weighed filter paper, dried in a vacuum oven at 65° C followed by storage in a desiccator over $CaCl_2$ for several days and then weighed. The work of Raistrick and that of Allen have both shown that the carbon content of fungus mycelia will vary; however, for these studies the carbon contents of all mycelia were calculated as 50 percent of the dry weight as suggested by Ward *et al.* (**1938**).

Respiratory carbon dioxide.—The CO_2 formed by the fungus was determined by titration of the contents of the two NaOH tubes with 1.000 normal HCl after precipitation of the carbonate with $BaCl_2$. In the work of Raistrick and his colleagues and also in the work of Allen, CO_2 which was dissolved in the culture medium was estimated by means of a Van-Slyke blood gas apparatus; however, such analyses were not made in the present instance, since the values reported by these previous workers were quite low, and while they would be significant in the preparation of a complete carbon balance, they are of little importance in our screening work.

After the above final analyses were made, sugar utilized was determined by calculating the difference between initial and final values. The weight of sugar utilized was then calculated to its equivalent weight of carbon. Similar conversions were made with the weights of mycelium and respired CO_2 so that all values reported are listed as grams of carbon. The percentage of carbon utilized which was converted to carbon of assimilation products of the mycelium and respiratory CO_2 was then calculated; the smaller this percentage, the greater the percentage of carbon which was converted to other metabolic products and the greater the potentiality of the organism as an agent of biosynthesis.

RESULTS

All data derived from the above analyses are presented in TABLE I (with the exception of pH and titratable acidity, which are handled in a

TABLE I

CONVERSION OF GLUCOSE CARBON TO ASSIMILATION AND CARBON DIOXIDE CARBON

No.	Species	Age (days)	Sugar utilized gm	Carbon as sugar utilized	Myce- lium formed	Carbon as mycelium	CO₂ evolved	Carbon as CO₂	Percent carbon recovered
190	Verpa conica	46	1.720	0.688	0.040	0.020	0.1848	0.0504	10
183	Omphalia chrysophylla	63	0.965	0.386	0.051	0.0255	0.0484	0.0132	10
137	Clitocybe monodelpha	42	1.410	0.564	0.052	0.026	0.0528	0.0144	12
189	Tricholoma albobrunneum	46	0.820	0.328	0.033	0.0165	0.0792	0.0216	12
165	Boletus cyanescens	42	0.835	0.334	0.033	0.0165	0.0396	0.0108	13
174	Agaricus placomyces	83	0.605	0.242	0.046	0.023	0.0308	0.0084	13
151	Peziza badia	54	6.926	2.770	0.260	0.130	0.5958	0.1625	15
147	Morchella deliciosa	42	0.805	0.322	0.029	0.0145	0.132	0.036	16
169	Boletinus merulioides	42	0.630	0.252	0.036	0.018	0.044	0.012	19
182	Mycena leaniana	63	0.645	0.258	0.060	0.030	0.066	0.018	19
145	Morchella crassipes	56	2.475	0.990	0.201	0.1005	0.3441	0.0938	20
91	Coprinus radians	42	3.370	1.348	0.219	0.1095	0.7876	0.2148	24
169	Boletinus merulioides	87	0.450	0.180	0.044	0.022	0.0836	0.0228	25
171	Lycoperdon gemmatum	76	1.125	0.550	0.146	0.073	0.2649	0.0722	26
180	Crepidotus latifolius	56	5.745	2.298	0.939	0.4695	0.6662	0.1817	28
162	Polyporus pennsylvanicus	46	1.065	0.426	0.079	0.0395	0.3080	0.0840	29
185	Pholiota acericola	46	1.185	0.474	0.136	0.068	0.2684	0.0732	30
178	Cortinarius armillatus	61	4.395	1.758	0.773	0.3865	0.5826	0.1587	31
152	Sclerotinia tuberosa	40	1.240	0.496	0.144	0.072	0.3124	0.0852	32

TABLE I—*Continued*

No.	Species	Age (days)	Sugar utilized gm	Carbon as sugar utilized	Myce- lium formed	Carbon as mycelium	CO_2 evolved	Carbon as CO_2	Percent carbon recovered
149	Morchella hybrida	52	7.335	2.934	1.384	0.692	1.0329	0.2817	33
126	Lepiota morgani	40	2.040	0.816	0.274	0.137	0.5808	0.1584	36
148	Morchella esculenta	40	1.820	0.728	0.192	0.096	0.6336	0.1728	37
150	Peziza venosa	52	2.490	0.996	0.218	0.109	0.9537	0.2601	38
138	Polyporus tsugae	40	1.675	0.670	0.311	0.1555	0.3995	0.1090	39
97	Hypholoma perplexum	42	1.855	0.742	0.348	0.174	0.4620	0.1260	40
159	Favolus canadensis	44	1.210	0.484	0.130	0.065	0.4677	0.1276	40
158	Ganoderma applanatum	44	1.800	0.720	0.338	0.169	0.5254	0.1433	43
144	Leotia lubrica	56	2.175	0.870	0.521	0.2605	0.4017	0.1096	43
155	Tremella sp.	46	1.393	0.5572	0.173	0.0865	0.5852	0.1596	44
154	Xylaria polymorpha	52	5.396	2.158	1.388	0.694	0.9621	0.2624	44
154	Xylaria polymorpha	44	4.755	1.902	1.207	0.6035	0.8650	0.2359	44
181	Hypholoma incertum	44	1.465	0.586	0.229	0.1145	0.5562	0.1517	45
170	Crucibulum vulgare	49	1.550	0.620	0.241	0.1205	0.6046	0.1649	46
100	Collybia velutipes	42	3.405	1.362	0.833	0.4165	0.8096	0.2208	47
120	Panus rudis	42	1.600	0.640	0.223	0.1115	0.7128	0.1944	48
123	Agaricus rodmani	54	1.045	0.418	0.286	0.143	0.2072	0.0565	48
175	Clitocybe illudens	50	2.195	0.878	0.504	0.252	0.6398	0.1745	49
185	Pholiota acericola	56	0.970	0.388	0.175	0.0875	0.3749	0.1022	49
184	Pluteus cervinus	56	0.420	0.1680	0.072	0.036	0.1720	0.0469	49

TABLE I—*Continued*

No.	Species	Age (days)	Sugar utilized gm	Carbon as sugar utilized	Myce- lium formed	Carbon as mycelium	CO_2 evolved	Carbon as CO_2	Percent carbon recovered
121	Coprinus atramentarius	40	1.175	0.470	0.171	0.0855	0.5896	0.1608	52
160	Polyporus lucidus	44	1.440	0.576	0.197	0.0985	0.7326	0.1998	52
142	Bulgaria rufa	59	1.130	0.452	0.300	0.150	0.3091	0.0843	52
177	Collybia radicata	61	1.900	0.760	0.435	0.2175	0.7150	0.1950	54
179	Cortinarius sp.	40	3.200	1.280	0.955	0.4775	0.8188	0.2233	55
94	Coprinus comatus	42	0.695	0.278	0.183	0.0915	0.2904	0.0792	61
188	Russula variata	40	2.375	0.950	0.441	0.2205	0.5298	0.1445	62
119	Morchella hybrida	49	1.045	0.418	0.253	0.1265	0.5562	0.1517	67
186	Pholiota praecox	56	0.380	0.152	0.109	0.0545	0.1764	0.0481	68
173	Amanita caesaria	50	1.460	0.584	0.481	0.2405	0.6468	0.1764	71
42	Bulgaria rufa	42	0.360	0.144	0.098	0.049	0.1936	0.0528	71
168	Boletus indecisus	26	1.415	0.566	0.862	0.431	0.2869	0.0782	90
166	Boletus luridus	61	0.790	0.316	0.307	0.1535	0.4985	0.1360	92
141	Bulgaria inquinans	52	0.360	0.144	0.175	0.0875	0.1719	0.0469	93

separate section). In this table, the fungi are listed in the order of increasing percentage of carbon utilized which is converted to mycelium carbon and CO_2 carbon. Thus, *Verpa conica* appears first in the table, since it converted only 10 percent of the carbon of the sugar utilized to mycelium carbon and CO_2 carbon; *Bulgaria inquinans* is listed last, since of the carbon utilized, 93 percent was recovered as mycelium and CO_2 carbon. When two or more species converted the same percentage of utilized substrate carbon to mycelium and CO_2 carbon, they were arranged in the order of decreasing average daily glucose utilization.

Economic coefficients.—From the results presented in TABLE I, it

FIG. 2. A comparison of the economic coefficients of fungi with the percentages of utilized substrate carbon which they converted to mycelium carbon and carbon dioxide carbon.

is evident that the simple methods described earlier provide a good index
to the biosynthetic potentialities of fungi at least to the extent that they
give some idea of the quantities of metabolic products (other than myce-
lium constituents and carbon dioxide) which may be expected from cer-
tain fungi. Although these methods are simple ones, it was thought that
perhaps economic coefficients might provide an even more easily deter-
mined index. For that reason economic coefficients (gms of glucose
utilized during the formation of 1 gm of mycelium) were calculated from
columns four and six of TABLE I. The results of these calculations are
presented in FIG. 2 where they are compared with the percentages of
carbon recovered as mycelium and carbon dioxide. From the figure it
is obvious that in general those fungi which convert but a small percentage
of the sugar carbon to mycelium and carbon dioxide carbon have high
economic coefficients, whereas those which convert large percentages have
much smaller economic coefficients. This relationship, however, is true
only of the test organisms as a group, and a more accurate index to the
quantitative biosynthetic potentialities of a fungus is obtained if the
amount of CO_2 evolved is measured and also taken into account. A case
in point is provided by a comparison of *Verpa conica* (#*190*) with
Omphalia chrysophylla (#*183*). Both of these organisms convert only
10 percent of the carbon of the sugar utilized to mycelium and CO_2 carbon,
and hence both fungi appear to be of equal promise (at least on a purely
quantitative basis) in a search for large amounts of metabolic products.
Calculation of their economic coefficients reveals that *V. conica* has an
E.C. of 43 while that of *O. chrysophylla* is only 18.9—an indication that
the latter species is more efficient in the conversion of substrate carbon to
tissue carbon. If economic coefficients alone were considered, *V. conica*
would probably be judged the species most promising for further in-
vestigation of large quantities of metabolic products, whereas actually both
species are apparently nearly equal in this respect. The discrepancy
between economic coefficient value and percentage of carbon converted to
other metabolic products is easily explained if CO_2/ mycelium ratios are
calculated: *V. conica* formed 4.62 gm of CO_2 per gm of mycelium while
O. chrysophylla formed only 0.95 gm of CO_2 per gm of mycelium.

Acid production.—While no attempt to determine the nature of the
various metabolic products was made during the course of this work,
initial and final pH readings and initial and final titratable acidities were
routinely made, and the values obtained provide some index as to whether
or not a considerable portion of the metabolic products are acidic. On the
whole, it can be stated that the organisms tested are not strong acid
formers, at least not in the sense that certain species of *Aspergillus* and

Penicillium are. Of the fungi studied, only sixteen formed acidic prod-
ucts in amounts equivalent to 0.5 ml or more of normal acid per 250 ml
culture. Greatest acid formation occurred in the culture of *Clitocybe
illudens* (#*175*) in which a total of 2.08 ml of normal acid was formed.
Calculated as acetic acid this quantity represents only 91 mg of which 36.4
mg is carbon. Since in the course of the 50-day incubation period 2.195
gm of glucose was utilized (878 mg of carbon), 36.4 mg of carbon rep-
resents only a 4.1 percent conversion of sugar carbon to acidic metabolic
products carbon. The 16 organisms which formed acid in greatest
amounts are listed in TABLE II in the order of decreasing acid formation.

TABLE II

PRODUCTION OF ACID BY HIGHER FUNGI

No.	Species	pH		Titratable acidity		Ml normal acid formed 250 ml culture
		Init.	Final	Init.	Final	
175	C. illudens	6.2	2.9	3.45	7.60	2.08
154	X. polymorpha	6.5	2.6	4.30	8.00	1.85
179	Cortinarius sp.	6.2	3.9	4.30	7.85	1.73
100	C. velutipes	6.3	2.7	4.20	7.50	1.65
151	P. badia	6.3	5.0	4.08	7.00	1.46
145	M. crassipes	6.3	4.7	4.08	7.00	1.46
188	R. variata	6.2	2.6	4.30	6.90	1.30
152	S. tuberosa	6.3	2.9	4.10	6.60	1.25
123	A. rodmani	6.3	3.2	4.08	6.15	1.04
173	A. caesaria	6.2	3.8	3.45	5.30	0.93
171	L. gemmatum	6.2	2.5	3.45	5.15	0.85
170	C. vulgare	6.2	3.1	4.30	5.80	0.75
138	P. tsugae	6.2	3.3	4.30	5.70	0.70
148	M. esculenta	6.3	3.3	4.10	5.50	0.70
169	B. merulioides	6.2	4.8	3.45	4.60	0.58
176	C. platyphylla	6.2	4.8	3.45	4.50	0.53

Actual amounts of carbon converted to metabolic products.—In TABLE
I the fungi utilized in these studies are listed in the order of increasing
percentage of substrate carbon converted to mycelium carbon and carbon
dioxide carbon and hence are listed in the order of decreasing percentage
of substrate carbon converted to other metabolic products. Thus, *Verpa
conica* (#*190*) and *Omphalia chrysophylla* (#*183*) which are listed
first in TABLE I appear to have greater potentialities as agents of bio-
synthesis than the fungus species subsequently listed. This is certainly
the situation if percentage alone is considered; however, in terms of actual
quantities of carbon converted to other metabolic products the test fungi
used here may be arranged in a quite different fashion. For this ap-
praisal, the total quantity of substrate carbon converted to compounds

other than carbon dioxide and mycelium constituents was divided by the number of days of incubation, thus yielding average daily carbon conversion values which could be compared directly. The values so obtained (average mg of substrate carbon converted per day to compounds other than CO_2 and assimilation compounds) varied over a wide range. The lowest value was 0.18 mg/day for *Bulgaria inquinans* (#141), which was listed last in TABLE I and hence appears to have the least potentialities whether judged on a percentage basis or on the basis of actual quantities of substrate carbon converted. The highest value was 45.8 mg/day for *Peziza badia* (#151) which was rated seventh in the list in TABLE I. The fifteen fungi which converted the greatest average daily

TABLE III

AVERAGE DAILY CONVERSION OF SUBSTRATE CARBON TO METABOLIC PRODUCTS
OTHER THEN MYCELIUM COMPOUNDS AND CARBON DIOXIDE

No.	Species	Mg carbon converted/day	Numerical position in Table I
151	*Peziza badia*	45.8	7
149	*Morchella hybrida*	37.7	20
180	*Crepidotus latifolius*	29.4	15
154	*Xylaria polymorpha*	26.9	30
154	*Xylaria polymorpha*	24.1	31
91	*Coprinus radians*	24.1	12
178	*Cortinarius armillatus*	19.7	18
100	*Collybia velutipes*	17.2	34
188	*Russula variata*	14.6	46
179	*Cortinarius* sp.	14.4	44
145	*Morchella crassipes*	14.2	11
190	*Verpa conica*	13.4	1
126	*Lepiota morgani*	13.0	21
137	*Clitocybe monodelpha*	12.4	3
150	*Peziza venosa*	12.0	23

quantities of substrate carbon to other metabolic products are listed in TABLE III, in order of decreasing amounts of carbon converted to these metabolic products.

DISCUSSION

As might be expected in any series of screening tests in which one particular property or capacity is measured for each individual species of a large group of organisms, considerable differences were manifest in the relative percentages of utilized substrate carbon which were converted to assimilation carbon and carbon dioxide carbon. Of the species examined, *Verpa conica* and *Omphalia chrysophylla* apparently have the capacity to convert the greatest percentages of substrate carbon to metabolic products other than carbon dioxide and the myriad relatively insoluble compounds (assimilation compounds) of which living mycelium

is composed. Since with both of these species only 10 percent of the carbon utilized was recovered as mycelium and carbon dioxide, the remaining 90 percent was unquestionably converted to another compound or compounds, some or all of which might conceivably be of considerable academic or economic importance. From the standpoint of large percentages of metabolic products both of these species synthesize them in proportions sufficiently large to make their isolation and identification relatively easy as compared perhaps with *Bulgaria inquinans* (*#141*), in which species 93 percent of the carbon of the sugar utilized was recovered as mycelium and carbon dioxide, leaving only 7 percent (in this particular instance only 9.6 mg of carbon) available for the synthesis of other metabolic products.

In view of the data presented in TABLE III, it should be noted that some caution should be exercised in attempting to evaluate quantitatively the metabolic potentialities of a fungus on the basis of percentage conversion alone. Thus, for a more accurate evaluation, percentage conversion should not form the sole basis for judgment, but actual quantities of substrate carbon converted should also be taken into account. In connection with this particular discussion, one further point should be noted. Thus far in this work the principal emphasis has been placed upon the quantities of metabolic compounds synthesized and little attention has been paid to the nature of these compounds. Quantity of metabolic products is undoubtedly of very considerable importance; however, in some instances quality of a metabolic product may be just as important as quantity and hence should not be overlooked.

The general picture which emerges from the work herein reported is that for studies concerning fungus metabolic products the higher fungi (*i.e.* those Ascomycetes and Basidiomycetes which develop relatively large, fleshy sporocarps) exhibit rather favorable possibilities. Of the fifty-three experiments reported here, in only three instances did the test fungi convert over 70 percent of the utilized substrate carbon to mycelium and carbon dioxide carbon. In thirty-nine instances the test fungi converted less than 50 percent of the substrate carbon to mycelium and carbon dioxide, which is indicative of the suitability of most of these organisms for further investigation of their metabolism. This observation is more or less in keeping with the findings of Raistrick and his colleagues from their studies of the metabolism of a large number of species of the "mold" type of fungus, since these workers reported that relatively few of the fungi which they studied yielded results which indicated that they should receive no further study. Our results, however, are not in agreement with those of Whitaker, who found that carbon dioxide and

mycelium were generally the only major end products of the 40 species of cellulolytic Basidiomycetes which he studied. If we have in our test organisms a fair sampling of the group, the observation must be that in general the potentialities of the higher fungi as biosynthetic agents are relatively high. It does appear, however, that the fleshy Ascomycetes as a group might be rather promising for more extensive investigation, since, although only 15 experiments involving species of Ascomycetes were conducted, eight species were rated in the top 50 percent (see TABLE I) on the basis of percentage conversion. Furthermore, of the fourteen species of fungi listed in TABLE III on the basis of their high average daily conversion of substrate carbon to metabolic products other than CO_2 and assimilation products, six are species of Ascomycetes.

In the course of the 53 experiments described above, in only three instances were duplicate runs made with the same fungus. The three species involved in replicated experiments were *Xylaria polymorpha, Pholiota acericola* and *Boletinus merulioides* (Nos. *154, 185* and *169* respectively). With respect to percentages of utilized substrate carbon converted to carbon recovered as mycelium and carbon dioxide, very good agreement was obtained between duplicate experiments conducted with *X. polymorpha* (44 percent in both instances), less close agreement between results obtained from duplicate experiments with *B. merulioides* (19 and 25 percent recoveries), and least close agreement occurred between the results of duplicate experiments with *P. acericola* (30 and 49 percent recoveries). The lack of close agreement encountered in the latter two species could possibly be interpreted as being due to some inherent variability within these two isolates; however, it is more probable that the discrepancies can be attributed to the fact that duplicate experiments were run at different times, and slightly different conditions of temperature and degree of aeration may have prevailed. Both *Morchella hybrida* and *Bulgaria rufa* are also listed twice in TABLE I, but in both instances different strains of the same species rather than duplicate experiments with the same fungi are involved. In the case of the two strains of *M. hybrida, #119* converted 67 percent of the substrate carbon to carbon dioxide and mycelium while *#149* converted only 33 percent to such materials. Smaller differences (*71* and *52* percent) were found between strains of *B. rufa* (*#42* and *#142*). These differences might also be attributed to possible differences in environmental conditions, but, since physiological differences between strains of the same fungus species seem to be the rule rather than the exception, it seems more likely that these differences are due primarily to inherent differences between strains.

A comparison of the range of economic coefficients of the test organ-

isms with the range of percentages of utilized substrate carbon converted
to carbon dioxide and mycelium showed that for the entire group of test
organisms the following general relationship exists: those fungi with high
economic coefficients usually converted relatively low percentages of sub-
strate carbon to mycelium and carbon dioxide, while in general the
reverse was true of the fungi with low economic coefficients. The cor-
relation was not a close one and it was felt that simple calculation of an
economic coefficient did not provide an adequate index to the poten-
tialities of an organism. The complicating factor is in the fact that
differences exist between different species in the amounts of carbon dioxide
formed during the formation of some unit weight of mycelium. For
example, *Cortinarius armillatus, Collybia radicata* and *Morchella hybrida*
(Nos. *178, 177* and *119*) have economic coefficients of closely similar value
(4.54, 4.36 and 4.13 respectively); however, they differ widely with
respect to the amount of substrate carbon converted to mycelium and
carbon dioxide (31, 54 and 67 percent). Such differences, of course,
reflect differences in conversion efficiency that exist between different
species. In order to determine the magnitude of such differences the ratio
of carbon dioxide carbon to mycelium carbon was calculated for each
species. This ratio was found to vary from 0.37 for *Agaricus placomyces*
(#*174*) to 5.36 for *Bulgaria inquinans* (#*141*). From such ratios
there emerges a somewhat different concept of the overall cellular meta-
bolic efficiency of fungi from that which emerges from similar calculations
based on Raistrick's data. With the species that Raistrick and his co-
workers studied the ratios of carbon dioxide carbon to mycelium carbon
varied from 0.81 to 13.9—a much wider range than that found in the
present work. This wider range, however, is not nearly as significant as
the distribution of species within the range. In our own studies nearly
91 percent of the fungi investigated had CO_2 carbon/mycelium carbon
ratios whose values were less than 2.0, while only 36 percent of the fungi
examined by Raistrick had ratios this small. Over 50 percent of our
fungi had ratios less than 1.0, while only two of over 240 species examined
by Raistrick had ratios this small. On this basis it seems probable that
the fleshy Ascomycetes and Basidiomycetes are considerably more effi-
cient than Penicillia, Aspergilli and Imperfect Fungi, if one assumes that
the less carbon dioxide that is evolved per unit weight of mycelium formed,
the more efficiently the organism is utilizing its energy in conducting
the various other processes of metabolism.

Calculations of similar ratios from Whitaker's data yielded for the
wood-destroying Basidiomycetes which he studied a range of CO_2
carbon/mycelium carbon ratios of 0.65 to 4.82—a range very close to

that obtained in the present work. Distribution within the range was different, since 59 percent of the species had ratios less than 2.0, while only 10 percent had ratios less than 1.0.

A somewhat surprising finding in the light of the many studies concerned with acid synthesis by various "molds," was that acidic metabolic products are apparently synthesized in exceptionally small quantities by the test organisms used in the present study. As Foster (**1949**) has pointed out, fungi are highly aerobic organisms and hence it would be logical to expect that among their products of metabolism such products of oxidation as the organic acids would be found. Since acid synthesis seems to represent but a very small portion of the overall metabolic processes of the fungi studied, it seems altogether possible that the nature of fleshy Ascomycete and Basidiomycete metabolism may differ in some very fundamental ways from the type of metabolism apparently of rather general occurrence in the "mold" type of fungi. In addition to this, the above finding serves to point out the possible fallacy of making wide generalizations concerning the physiology of fungi which are based solely upon studies of one particular group or type of fungus.

SUMMARY

Fifty-three separate experiments were conducted with fifty different strains and species of higher fungi. All experiments were conducted in such manner that the following analyses could be made: gms of glucose utilized, gms of carbon dioxide evolved and gms of mycelium formed; in addition to these analyses, changes in pH and titratable acidity of the culture medium were also measured. The results and conclusions may be summarized as follows:

1. An index to the biosynthetic potentialities of a fungus may be obtained by calculating the percentage of utilized substrate carbon converted to mycelium carbon and carbon dioxide carbon. To provide a more accurate index, however, the average per day conversion of substrate carbon to carbon of other metabolic products should also be taken into account. This latter, however, represents additional calculations rather than additional analyses.

2. In general, economic coefficient values vary inversely with percentages of substrate carbon converted to mycelium carbon and carbon dioxide carbon; however, this relationship does not represent a close correlation, and economic coefficient alone is not considered an adequate basis for evaluation of the biosynthetic potentialities of a fungus.

3. The Ascomycete and Basidiomycete species studied were shown

to vary quite widely in their biosynthetic potentialities when these were judged on the basis of percentage of utilized substrate carbon converted to assimilation carbon and carbon dioxide carbon. Thus, with *Verpa conica* only 10 percent of the carbon of the sugar utilized was recovered as mycelium and carbon dioxide while with *Bulgaria inquinans* 93 percent was so recovered.

4. As a group the test organisms are considered to have rather high potentialities, since in 39 out of 53 experiments these fungi converted less than 50 percent of the utilized substrate carbon to mycelium and carbon dioxide.

5. When CO_2 carbon/mycelium carbon ratios were calculated, they were found to vary from 0.37 to 5.36—a range closely approximating that found by Whitaker in his work with wood-rotting Basidiomycetes but much more narrow than that found by Raistrick and his co-workers in their studies of a large number of fungi of the "mold" type. More significant than this difference in range, however, was the distribution of fungi within the range. In the present study over 90 percent of the test organisms had ratios less than 2.0, and over 50 percent had ratios less than 1.0.

6. Acid metabolic products were formed in extremely small quantities by all of the organisms studied—a finding which leads to the suggestion that the type of metabolism existent in these fungi may be fundamentally different from that exhibited by many of the much-studied fungi of the "mold" type.

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THE PRESERVATION OF DERMATOPHYTES
AT SUB–FREEZING TEMPERATURES

ESTHER MEYER

In most laboratories stock culture collections of dermatophytes on Sabouraud's dextrose agar slants are stored at room temperature or in a refrigerator at 5° C to 10° C. To insure viability, subcultures must be prepared every few months. Dermatophytes are particularly subject to pleomorphism, degeneration or mutation (whichever one may choose to call it) usually assuming a vegetative character evidenced by white, fluffy, non-sporulating aerial mycelia. Such alterations are most likely to occur after prolonged cultivation and frequent transfers. Some, such as *Microsporum canis, Epidermophyton floccosum, Microsporum gypseum* and *Trichophyton mentagrophytes* become pleomorphic after relatively few subcultures.

Sabouraud (**11**) was aware of these changes and suggested the use of "conservation" medium to maintain stock collections. Its use has been discontinued, since experience over the years has shown it does not control pleomorphism to an appreciable degree.

Lyophilization has been used to preserve some fungi but in our experience, and also that of Ajello, Grant and Gutzke (**1**), dermatophytes as a rule do not survive this treatment. Ajello and associates (**1**) recommended storage of pathogenic fungi under mineral oil but found this did not prevent pleomorphic changes in all dermatophytes.

As early as 1918, Barthel (**4**) suggested the use of sterile soil to preserve yeasts. Thom and Raper (**12**) cited several papers in which Aspergilli, Penicillia and other saprophytic fungi were maintained on this medium. Ciferri and Redaelli (**5**) stored three species of dermatophytes on soil for three years and observed little pleomorphic change in transplants. Bakerspigel in 1953 (**2**) and in 1954 (**3**), after comparing loam, clay, peat, sand, sawdust and kieselguhr for the storage of a variety of pathogenic and saprophytic fungi, found loam to be the most satisfactory. Included were 7 species of dermatophytes, all of which remained viable for one year and 3 for as long as two years. Subcultures of these prepared from seeded loam were in their "wild" or primary state while controls maintained on Sabouraud's dextrose agar became pleomorphic.

Lindner (8), Luyet (9) and Kadisch (6, 7) performed experiments on the effect of exposing saprophytic species and a few dermatophytes to temperatures of $-8.9°$ C to $-272°$ C. They have shown that these fungi often resisted temperatures of $-20°$ C to $-30°$ C or even $-272°$ C for varying periods. They pointed out that for some organisms there are zones of lethal temperatures, one above zero, one slightly below zero and then one of non-lethal temperatures extending to $-272°$ C. These studies were done primarily to observe the effect of low temperatures on viability rather than as a method of storage.

It has always been difficult to maintain typical cultures for teaching purposes throughout the year. In search of a convenient, simple routine to overcome this obstacle in particular, it seemed worth while to further investigate the storage of fungi at low temperatures. To us it is more important to have viable strains in typical form as required and less important that stored cultures survive for very long periods of time. Consequently such a study was undertaken and has been in progress for the past two years.

It is the purpose of this paper to present the results of our work, which involved the storage of dermatophytes at sub-freezing temperatures for various periods of time. To our knowledge, the technique about to be described has not been used previously.

MATERIALS AND METHODS

Typical cultures of dermatophytes grown on Sabouraud's dextrose agar were obtained for this study. Proom and Hemmons (10) investigated the effect of freezing on the viability of various bacterial cultures and demonstrated the best survival rates occurred when they were suspended in protective colloids as papain digest broth, serum or gelatin. We selected, therefore, human blood plasma and litmus milk as suspending vehicles for the dermatophytes under investigation.

We found it convenient to use sterile, cotton-stoppered tubes four inches long and one-half inch in diameter for this work. Bits of growth from each culture were removed with a sterile, hooked wire and transferred to tubes containing 1.5 ml of sterile human plasma and 1.5 ml of sterile litmus milk. These were stored at the arbitrary temperatures of $-22°$ C and $-52°$ C for varying periods of time up to two years. As needed, frozen preparations were thawed, a few loopfuls of each transferred to a Sabouraud's dextrose agar slant and incubated at 28° C. Subsequently each thawed specimen was refrozen and later cultured to determine whether the fungus remained viable and typical.

RESULTS

Twelve species of dermatophytes with a total of 61 isolates were included in this study (Table I). Three isolates (*Epidermophyton floccosum 210, Trichophyton violaceum 517* and *Trichophyton tonsurans A57*) failed to survive this method of storage. Apparently this is not characteristic of these species since other isolates of the same species

TABLE I

DERMATOPHYTES SUSPENDED IN HUMAN BLOOD PLASMA OR LITMUS MILK AND
STORED AT SUB-FREEZING TEMPERATURES

Periods of viability up to two years

Genera and species	Number of isolates	
[1] *Microsporum*		
audouini	8	
canis	8	
gypseum	3	
[1] *Epidermophyton*		
floccosum	5	
floccosum	1	(210) [3]
[1] *Trichophyton*		
mentagrophytes	10	
rubrum	10	
megnini	1	(Dr. Elizabeth Hazen)
schoenleini	2	(C.D.C. 197, A408)
schoenleini	2	
concentricum	1	(Mrs. C. L. Taschdjian)
ferrugineum	1	(Mrs. C. L. Taschdjian)
violaceum	4	(C.D.C. 517,[2] J.B., A3, A577)
tonsurans	5	(C.D.C. 608, 389, A57,[2] A63, 517)

[1] Unless indicated otherwise, cultures were isolated in this laboratory.
[2] Not viable at end of four months of storage.
[3] Not viable at end of twelve months of storage.

survived. All transplants were identical in gross and microscopic characteristics to the original cultures with no pleomorphic changes discernible. Surprisingly, the lag growth period was shorter than in the controls prepared from stock collections. We found that some species did not survive the thawing and refreezing treatment and therefore recommend that thawed cultures be discarded and fresh preparations made for storage purposes.

DISCUSSION

As mentioned previously, the preservation of a limited number of dermatophyte species on soil was demonstrated by Ciferri and Redaelli

(5) and Bakerspigel (2, 3). These workers introduced a useful method. However, as pointed out by Zeidberg (13), soils are very complex mixtures of varying composition and these biologically complex substrates are difficult to standardize.

We think the method of preservation described in this paper offers certain advantages. First in importance, pleomorphism did not occur in the dermatophytes when stored either at − 22° C or − 52° C. Cultures of dermatophytes which ordinarily do not survive long storage at temperatures of 5° C to 10° C, did remain viable in our experiments. The suspending menstrua, sterile human plasma or litmus milk, and the equipment required are readily available in most laboratories. The routine is simple and rapid.

SUMMARY

Viable cultures of dermatophytes free from pleomorphic changes were maintained for as long as two years by suspending the fungi in the protective colloids, human blood plasma or litmus milk, and storing at either − 22° C or − 52° C. The routine is simple, convenient and expedient. This method of storage relieves the mycologist of the laborious task involved in the periodic transfer of stock collections.

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ASCOCARPS OF ASPERGILLUS AND PENICILLIUM [1]

CHESTER R. BENJAMIN [2]

(WITH 21 FIGURES)

The industrial and commercial importance of *Aspergillus* and *Penicillium* has stimulated extensive study of these form genera. According to Raper and Thom (**1949**), more than 700 species have been described as penicillia alone. The emphasis in current literature is on the conidial stages, and the manuals by Raper and Thom (**1949**) and Thom and Raper (**1945**) have done much to alleviate the pressing taxonomic needs regarding those imperfect stages. Those species forming ascocarps, however, must, in accordance with the International Rules of Nomenclature, be placed among the Ascomycetes and assigned to ascomycetous genera. This fact has been accepted by the majority of mycologists, but in practice has been extremely difficult because of the taxonomic confusion and paucity of comparative data on these ascocarpic forms. It is therefore proposed to examine the comparative morphology of the ascocarps of typical species of both *Aspergillus* and *Penicillium*, as well as some of the pertinent generic taxonomy.

The genus name *Aspergillus* dates back to Micheli (**1729**), who coined the term because of the similarity between the conidial head and a holy water sprinkler. Link (**1809**) first used the names *Penicillium* and *Eurotium*, the former as an imperfect, and the latter as a perfect genus. Fries (**1821**) recognized all three genera, and DeBary (**1854**) reported the connection of *Aspergillus* and *Eurotium*. Berkeley (**1857**) erected the genus *Emericella* for the new species *E. variecolor* Berk. & Br. The inclusion by Brefeld (**1874**) of the perfect stage in his study

on *Penicillium* prompted some authors to include that genus in the Ascomycetes. Ludwig (**1892**), for example, discussed *Penicillium* as *Eupenicillium crustaceum* (L.) Fr. (*P. glaucum*). The name he used seems to be of doubtful application and is considered here as possibly synonymous with the genus *Carpenteles,* which was erected by Langeron (**1922**) for the perfect stages of penicillia of the *P. glaucum* type. Langeron also proposed the genus *Diplostephanus* as an ascomycetous genus for species of the *Sterigmatocystis (Aspergillus) nidulans* type. Some five years later Vuillemin (**1927**) proposed the genus *Sartorya* for an ascomycetous species considered synonymous by Thom and Raper (**1945**) with *Aspergillus fischeri* Wehmer.

Thus it can be seen that some perfect stages of *Aspergillus* have been called *Eurotium, Emericella, Diplostephanus,* and *Sartorya.* There have been other names as well which, because of their evident synonymy, will not be considered here. The perfect stages of *Penicillium* have been assigned to *Carpenteles* or, perhaps more commonly, to no ascomycetous genus at all.

Comparison of the perfect stages of *Aspergillus* would seem to indicate that these forms comprise three fairly sharply defined series. These series can be correlated with the groupings made by Thom and Raper (**1945**), who used characteristics of the conidial apparatus as primary ones, but because of the differences in the development and in the morphology of the mature ascocarps, the three series are here considered to be of generic rank. One series is composed of those species in which the ascocarp wall is composed of a single layer of large cells; the ascocarp is tiny $(50-175\,\mu)$, yellowish at some stage of development, and loosely suspended in a network of hyphae encrusted with yellowish or reddish granules, and is initiated by a coiled ascogonium which often is fused with an antheridium. The ascospores of this series are lenticular and typically pulley-shaped, although the furrows are essentially absent in a few species. Following the species concept of Thom and Raper (**1945**), this series would contain the perithecial forms of their *Aspergillus glaucus* group, and is considered here as comprising the genus *Eurotium* Link.

A second series is composed of those forms in which the ascocarp wall is made up of several layers of narrow, interwoven hyphal strands; the ascocarp is larger (up to $500\,\mu$) and seemingly of much more indeterminate growth than in the first series, and is initiated by a curled ascogonium with no evidence of an antheridium. Ascospores of this series are frilled or banded and colorless. This series would include the forms classified by Thom and Raper (**1945**) in their *Aspergillus fischeri*

series, and are considered here as comprising the genus *Sartorya* Vuillemin.

The third series is composed of those species in which the ascocarp wall is made up of compacted, narrow, interwoven hyphae several layers thick; the ascocarp is dark-colored, fairly large (up to $450\,\mu$), and enveloped by large, globose, thick-walled "hülle" cells, and is initiated without differentiated ascogonia or antheridia. This series would in-

genus	ascocarp initials	young ascocarp	asci	ascospores
Eurotium (imperfect stage- Aspergillus)				
Sartorya (imperfect stage- Aspergillus)				
Emericella (imperfect stage- Aspergillus)				
Talaromyces (imperfect stage- Penicillium)				
Carpenteles (imperfect stage- Penicillium)				
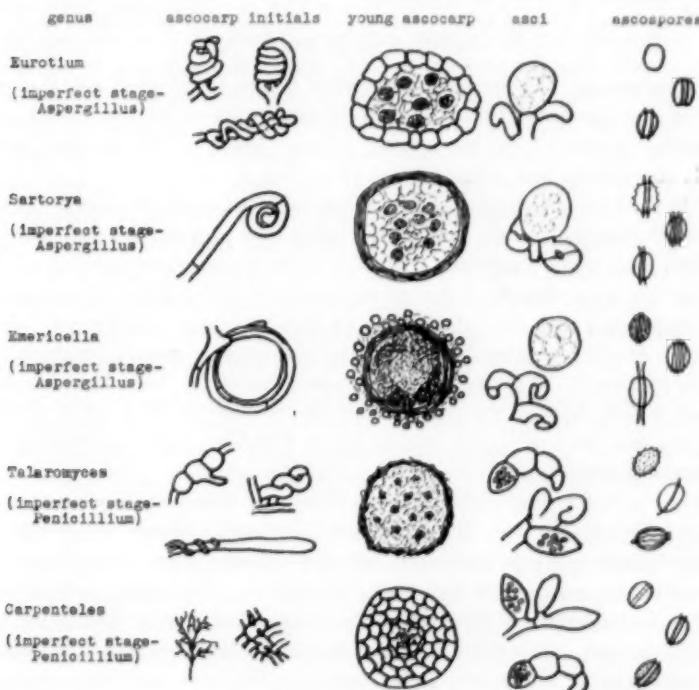
FIG. 1. Diagrammatic representation of the ascocarpic structures of the genera possessing *Aspergillus* and *Penicillium* conidial stages.

clude the perithecial forms treated by Thom and Raper (**1945**) as the *Aspergillus nidulans* group. Since the series would include both forms previously called *Emericella* Berk. & Br. and *Diplostephanus* Langeron, the latter genus is a synonym, and the group is considered here as comprising the genus *Emericella* Berkeley & Broome.

The salient features of the three series which possess an *Aspergillus-*

type conidial stage are diagrammatically represented in FIG. 1. The three genera are also easily distinguishable as in the following key:

I. Ascocarps naked, loosely suspended in a network of yellow or red hyphae. Ascocarp wall one layer thick, composed of large, polygonal cells....*Eurotium*

I'. Ascocarps invested with either sterile hyphae or "hülle" cells, borne discretely or in clumps on substrate surface. Ascocarp wall composed of several layers of interwoven hyphae compressed.............................II

    II. Ascocarp loosely invested with sterile hyphae, initiated by curled ascogonium. Ascospores hyaline................................*Sartorya*

    II'. Ascocarp invested with "hülle" cells. Ascocarpic initial undifferentiated. Ascospores reddish-purple or blue.....................*Emericella*

Comparison of a number of perfect stages of *Penicillium* was reported by Emmons (**1935**). His observations were confirmed and somewhat extended by Raper and Thom (**1949**). The present study tends to confirm and extend those preceding it.

On the bases of ascocarp structure and development, Emmons (**1935**) reported that the perfect stages of *Penicillium* represented two sharply defined lines of development. However, he found some overlap of the two series with respect to the arrangement of asci within the ascocarps and concluded that the information at that time was insufficient "for a revision of the genus or the creation of new generic names for the ascocarpic species." In the twenty years since that study, little has appeared which would support the inclusion of the two series in a single genus. The studies here reported justify, it is believed, their assignment to separate genera.

One series is made up of those forms of which the ascocarp wall consists of interwoven, but not fused, hyphae. These forms are of indeterminate growth and may increase in size after some asci and spores are mature. The perithecial initials are of variable morphology and show different degrees of differentiation of the ascogonia in the several species. In addition, there appear to be several points within the ascocarp at which the ascogenous hyphae and asci are initiated. The asci of this series are preponderantly borne in short chains from the ascogenous hyphae. The three forms examined in which the asci were not borne in chains were found to have crozier formation, which is thought to be indicative of the labile nature of the group. This labile nature is also indicated, perhaps, by the fact that in a few species the ascospores tend to be frilled rather than spiny, which is the central tendency. This series can be equated to the *Penicillium luteum* series of Raper and Thom (**1949**) and is considered here as comprising a new ascomycetous genus *Talaromyces*.

The other series is made up of those forms of which the ascocarp

wall is composed of extremely thick-walled, "sclerotia-like" cells. The exception is the perfect stage of *Penicillium levitum* Raper & Fennell, in which the ascocarp wall is firm, but of more parenchymatous nature than sclerotioid. The ascocarpic initials of this series consist of a group of modified cells in the crotch of a tree-like network of hyphae. The ascocarp matures from the center outwards after having reached a definitive size in the fashion reported by Emmons (**1935**). The asci are borne singly on short branches of the ascogenous hyphae except in one or possibly two species in which they are in short chains. The spores exhibit a range from almost complete absence of any furrow to the presence of a well-defined one. The developmental and mature morphology of the ascocarps seem to separate this series sharply from the species assigned to *Talaromyces,* notwithstanding the aberrance of a species or two in the arrangement of asci in chains. This series includes the few species which have been assigned by authors to the genus *Carpenteles* Langeron. That genus is considered here as valid and restricted to this sclerotioid series.

The salient features of the two series as presented by Emmons (**1935**) and supplemented by Raper and Thom (**1949**) and the present study are diagrammatically represented in FIG. 1. The two genera are also easily distinguishable as in the following key.

I. Ascocarps soft, of indeterminate growth, with wall composed of loosely to tightly interwoven hyphae. Usually yellowish or surrounded by hyphae encrusted with yellow or orange granules........................*Talaromyces*
I′. Ascocarps firm, of determinate growth, with wall of thick-walled sclerotioid or parenchymatous-like cells, ripening from center outward. Usually some color other than yellow or orange........................*Carpenteles*

Included in the present report are 29 strains representing 23 species of the 5 ascomycetous genera. Of these, 14 strains are subcultures of type material. The others are typical strains of forms already described in the literature. Inasmuch as the conidial stages of all forms studied are adequately treated by Raper and Thom (**1949**) or Thom and Raper (**1945**), they are not treated here, and the discussions of the ascocarps are limited to such observations as are considered new, unstressed, or of some phylogenetic significance. The observations made are based either on study of fresh material or paraffin sections, or both.

EUROTIUM Link ex Fries, Syst. Myc. **3** : 331. 1829.

Type species: *Eurotium herbariorum* [Pers.] Link ex Fries, Syst. Myc. **3** : 332. 1829.

Conidial stage: *Aspergillus* Micheli ex Fries.

Eurotium repens De By. in DeBy. & Wor., Beitr. Morph. u. Phys.
   Pilze **3** : 1.   1870.   Figs. 2, 9

The imperfect stage of this species is represented by the form
described by Thom and Raper (**1945**) as *Aspergillus repens* (Cda.)
DeBary.   The ascocarps were studied from a culture received from the
Northern Utilization Research Branch of the U.S.D.A. as NRRL *52*.
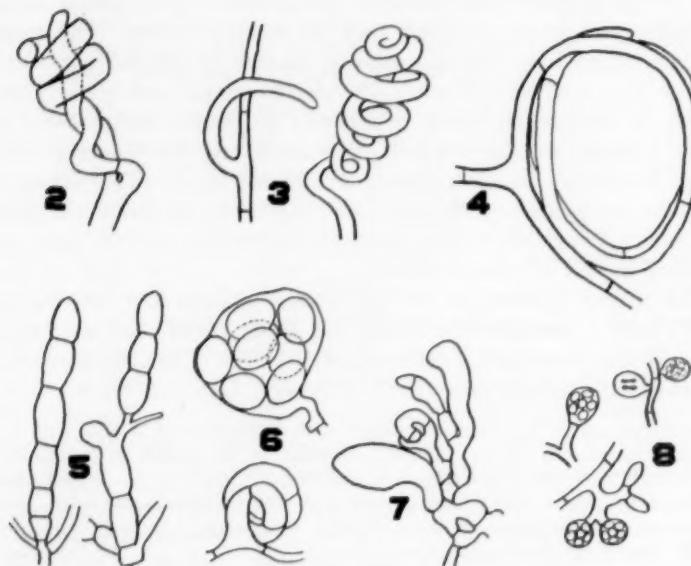
Figs. 2–8.   2. Ascogonium and antheridium of *Eurotium repens*, × 1100.   3.
Ascogonium of *Eurotium amstelodami*, showing very young and mature stages,
× 475.   4. Ascocarpic initial of *Emericella quadrilineata*.   The branch at the left
is the base of a conidiophore, × 1100.   5. The relatively undifferentiated ascogonia
of *Talaromyces rotundus*, × 1100.   6. Ascogonium and almost mature ascus of
Talaromyces striatus, × 1100.   7. Young ascus and ascogenous hyphae of *Talaro-
myces avellaneus*, showing croziers, × 1100.   8. Young asci and ascogenous hyphae
of *Carpenteles levitum*, × 690.

The development of the ascocarp seems to follow the process as
outlined by Dale (**1909**) and seems to be fairly typical for the genus.
Plates XXII and XXIII of Dangeard's (**1907**) study of *Eurotium her-
bariorum* Wigg. could serve almost equally well for *E. repens* also.
The ascocarp is initiated by a coiled ascogonium alongside of which
the antheridium grows and attaches at the apex.   These organs are soon

invested with a single layer of sterile hyphae which seems to originate from the same organ-bearing hypha and grow up around the developing ascogonium. These young developing ascocarps are quite distinctive, as are the mature ones, with their single-hypha stalks and walls composed of large, irregular cells which are characteristic of the genus. The asci of *E. repens* are formed by means of croziers from ascogenous hyphae and are scattered throughout the interior of the ascocarp. The asci (as well as some internal tissue and even portions of the wall cells) deliquesce, releasing the ascospores. The ascospores are lenticular, smooth-walled, and have little or no evidence of ridges or furrows.

**Eurotium carnoyi** (Thom & Raper) comb. nov.

> *Aspergillus carnoyi* Thom & Raper, U.S.D.A. Misc. Publ. 426, p. 34, 1941.

Aspergillus carnoyi Thom & Raper is a synonym only with respect to the ascocarpic stage. The name is valid for the conidial form. The strain used in the present study was NRRL *126*.

The morphology of the mature ascocarp of this species differs from that of *E. repens* in that the sizes of the ascocarp, asci, and ascospores are all about a third larger than in *E. repens*, and thus approximate those of *E. echinulatum* Delacr. which, however, has ascospores that are very definitely furrowed and ridged, whereas those of *E. carnoyi* are not. Conidial, physiological, and cultural differences as described by Thom and Raper (**1945**) would also seem to indicate that the species is distinct. Because of cultural difficulties, the ascocarpic initials were not ascertained in this study nor have they previously been described.

EUROTIUM ECHINULATUM Delacr., Bull. Soc. Myc. France **9**: 266, pl. XIV, fig. III. 1893. FIG. 13

The imperfect stage of this species is represented by *Aspergillus brunneus* Delacr. Descriptions of both the ascocarpic and conidial stages are given by Thom and Raper (**1945**) under the name *Aspergillus echinulatus* (Delacr.) Thom & Church. Subcultures of their strain, NRRL *131*, were studied by the present writer with confirmatory results. The morphology of the mature ascocarp differs little from that of *E. repens* and *E. carnoyi*. The ascocarp wall is composed of the same type of large polygonal cells arranged in a single layer. The ascocarp itself is but slightly larger than that of most other members of the genus. The asci and ascospores, on the other hand, are enormous when com-
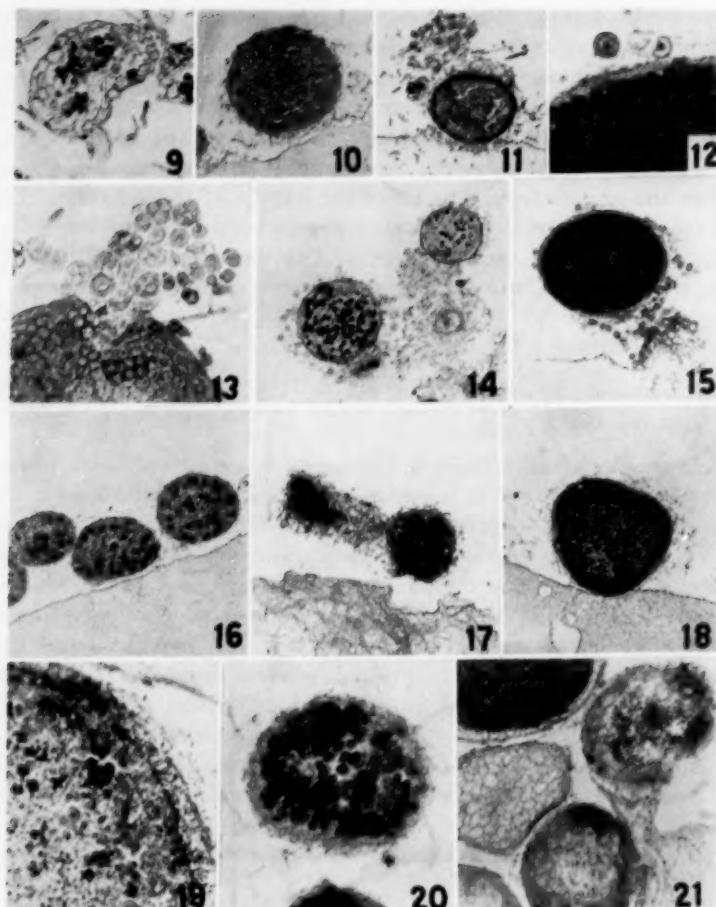
Figs. 9–21. 9. *Eurotium repens:* section of an almost mature ascocarp, × 200. 10. *Sartorya fumigata:* section of an ascocarp filled with ascospores, × 70. 11. *Emericella nidulans:* section of an ascocarp filled with ascospores, × 70. 12. *Emericella variecolor:* Section of an ascocarp showing wall structure, × 285. 13. *Eurotium echinulatum:* a portion of a crushed ascocarp with asci extruded, × 215. 14. *Emericella rugulosa:* section showing ascocarps in various stages of maturity, × 70. 15. *Emericella variecolor:* section of an ascocarp filled with ascospores, × 70. 16. *Talaromyces wortmannii:* section of ascocarps, showing dark, fertile areas and scanty wall, × 50. 17. *Talaromyces luteus:* section showing loose construction of the ascocarp wall, × 70. 18. *Talaromyces rotundus:* section of the ascocarp, × 50. 19. Same as Fig. 18, enlarged, showing asci and undifferentiated wall, × 200. 20. *Talaromyces striatus:* section of the ascocarp, × 285. 21. *Carpenteles javanicum:* section showing several stages of ascocarp development, × 200.

pared to species such as *E. amstelodami*, a characteristic which in the past has led to the practice of grouping the large-spored forms together and the small-spored forms together. If, when all the species have been studied developmentally, it is still found necessary to use spore characters as criteria of infrageneric relationships, perhaps the characteristics of the spore wall would be more fundamental. The spores of the various species seem to form a well-defined series from the smooth, unfurrowed ones of *Eurotium repens* and *E. carnoyi* through the completely roughened, prominently furrowed and ridged ones of *E. amstelodami*.

EUROTIUM MEDIUS Meiss., Bot. Zeit. **55**: 356.   1897.

The imperfect stage of this species is represented by *Aspergillus medius* Meiss. Both the perfect and imperfect stages were described by Meissner (**1897**) and by Thom and Raper (**1945**). Strain NRRL *124*, on which the descriptions by Thom and Raper were based, was used for the present study. Observations were confirmatory and need not be repeated here.

EUROTIUM AMSTELODAMI Mangin, Ann. Sci. Nat. Bot. IX. **10**: 360. 1909.   FIG. 3

The imperfect stage of this species is *Aspergillus amstelodami* (Mangin) Thom & Church. The study was made from cultures of GWM *6349* representing material isolated from dead stems of a convolvulaceous plant collected near Salinas, Ecuador. The perithecial initial seemed to arise as a side branch of a hypha. This young ascogonium soon started to coil, making about 5–7 turns. The basal portion of the coil seemed much more loose than the apical portion, looking somewhat different from *E. repens* in that respect. Also, nothing which could be called an antheridium was observed. Dale (**1909**) reported that in *E. repens* the antheridium was present only about half the time. The tendency in this genus, then, is perhaps toward the loss of the antheridium. If that is the case, *E. amstelodami* would represent a more advanced member of the genus phylogenetically. This position would seem to be supported by the fact that the ascospores are rough over their entire surfaces and have pronounced furrows and ridges in the equatorial regions, and by the paucity or lack of encrusted sterile mycelia. The small size of the ascospores would, then, be either a manifestation of reduction or evidence of evolution proceeding in two directions in the genus.

Sartorya Vuillemin, Compt. Rend. Acad. Sci. (Paris) **184**: 136. 1927.

> Type species: *Sartorya fumigata* Vuill., Compt. Rend. Acad. Sci.
> (Paris) **184**: 136. 1927.
> Conidial stage: *Aspergillus* Micheli ex Fries.

Sartorya fumigata Vuill., Compt. Rend. Acad. Sci. (Paris) **184**:
136. 1927. Fig. 10

The imperfect stage of this species is *Aspergillus fumigatus* Fres. Descriptions of both the conidial and perithecial forms are included by Thom and Raper (**1945**) under the name *Aspergillus fischeri* Wehmer. Subcultures of their strain (NRRL *181*) were used for the present study. The development of the perithecium had been described earlier by Olive (**1944**).

The perithecial initials are readily observable in young cultures as upright branches with curled tips. This "curling" is distinguished from "coiling" in that the former is essentially in the same plane as the uncoiled portion of the organ rather than at right angles to it, as is more nearly the case of the latter type which seems more characteristic of the genus *Eurotium*. The ascocarps are considerably larger than those found in species of *Eurotium* and are of much more indeterminate growth. The ascocarp wall is composed of interwoven hyphal strands, more or less compressed, and of 2–3 layers even in mature specimens. The asci do not seem to differ materially from those of *Eurotium*, and as in that genus are formed from ascogenous hyphae by croziers. The spores are frilled rather than furrowed.

Although *S. fumigata* was the only species observed, this genus probably includes other forms on the basis of described perithecial characteristics. The forms described by Yuill (**1953**) as *Aspergillus quadricinctus*, by Lindt (**1889**) as *A. malignus*, and by Fennell and Raper (**1955**) as *A. aureolus* would certainly be included, and perhaps even those forms described by Raper *et al*. (**1953**) as *A. citrisporus* von Höhn. emend. and as *A. ornatus*, although the nature of the ascocarp wall would seem to exclude them.

Emericella Berk. & Br., in Berk., Introd. Crypt. Bot. 340. 1857.

> Type species: *Emericella variecolor* Berk. & Br., in Berk., Introd.
> Crypt. Bot. 340. 1857.
> Conidial stage: *Aspergillus* Micheli ex Fries (or, more narrowly,
> *Sterigmatocystis* Cramer).

EMERICELLA VARIECOLOR Berk. & Br., in Berk., Introd. Crypt. Bot.
340, fig. 76. 1857. FIGS. 12, 15

The imperfect stage is represented by the form described by Thom
and Raper (**1945**) as *Aspergillus variecolor* (Berk. & Br.) Thom &
Raper. Three cultures were used for the present study: NRRL *212*,
representing *E. variecolor* var. *variecolor;* GWM *6273* and GWM *6368*,
both representing **E. variecolor** var. **astellata** (Fennell and Raper)
comb. nov. The latter variety differs from the type primarily in ascal
and spore characteristics.

The ascocarp seems to originate from a loosely looped coil of hyphae
similar to those which give rise to the conidiophores. On a weak oat
medium the ascocarp is commonly formed at the base of a conidio-
phore. The hyphal system at this area seems to branch rather pro-
fusely, forming a clump of rather wide hyphae. Some of these branches
anastomose; others form intercalary or terminal swellings which enlarge
and eventually form the "hülle" cell covering of the ascocarp. The early
formation of these "hülle" cells obscures the early development of the
ascocarp. Sections of the very young ascocarp indicate that the wall is
composed of the wide, intertwined hyphae making up 2–3 layers. In
somewhat older sections the wide hyphae are less evident and are sur-
rounded by as many as 6–8 layers of narrow hyphae on which are borne
the "hülle" cells. At this stage of development there is still little
evidence of differentiation within the ascocarp. Sections of older asco-
carps show no evidence of the wide hyphae, the wall seemingly being
composed of 3–6 layers of the "hülle"-cell-bearing hyphae. These layers
are somewhat compressed at maturity. The young asci in *E. variecolor*
var. *astellata* seem unique in having a number of lobes. These lobes
are not as evident in the mature asci. The ascospores are very widely
frilled, and portions of the frills often extend into the lobes of the asci.

EMERICELLA NIDULANS (Eidam) Vuill., Compt. Rend. Acad. Sci.
(Paris) **184**: 137. 1927. FIG. 11

Sterigmatocystis nidulans Eidam, Cohn's Beitr. Biol. Pflanzen **3**:
392. 1883.

The imperfect stage of this species is the form described by Thom
and Raper (**1945**) as *Aspergillus nidulans* (Eidam) Wint. The species
is represented in the present study by NRRL *187;* GWM *5502*, isolated
from rabbit dung, Horsley, Surrey, England; and GWM *5503*, isolated
from cactus, Caliente, Nevada.

The development of this species would appear to be similar to that of *E. variecolor*. The supporting hyphae and "hülle" cells, however, do not form any stalk or pseudostalk as in the case of *E. variecolor*. Hülle cells seem less abundant than in that species, also. There has been in the past some confusion with respect to the thickness of the ascocarp wall in *E. nidulans*. Eidam (**1883**) described his form as having a fairly thick, firm wall. Thom and Raper (**1945**) have stated that the ascocarp wall of their strain was but one cell layer thick. Results of the present study, based on subcultures of the same strain used by Thom and Raper, clearly indicate that the wall is composed of several layers of cells except in the case of overmaturity, at which time the dissolution of the inner portion of the wall sometimes leaves at least portions of the wall as thin as one layer of cells. As a matter of fact, in all four species of *Emericella* included in this paper the ascocarp wall was made up of several layers of interwoven hyphae. As the ascocarps ripen, however, these hyphae become more compressed and in sections of overmature ascocarps, especially in the case of *E. rugulosa*, may be only one layer thick.

Emericella quadrilineata (Thom & Raper) comb. nov. Fig. 4

Aspergillus quadrilineatus Thom & Raper, Mycologia **31** : 660. 1939.

Aspergillus quadrilineatus Thom & Raper is a synonym only as regards the ascocarpic form. The name is valid for the conidial stage.

The present study was based on the strain GWM *6411*, which was found to differ little from the description as presented by Thom and Raper, except that the ascocarp wall was of several layers of interwoven hyphae somewhat compressed. The ascocarps appeared to be initiated by loops of relatively wide hyphae at the bases of conidiophores much as described under *E. variecolor*. Increase in size seemed to be by intercalary growth of wall hyphae. The number of "hülle" cells seemed relatively small.

Emericella rugulosa (Thom & Raper) comb. nov. Fig. 14

Aspergillus rugulosus Thom & Raper is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Both stages are described by them in Mycologia **31** : 661. 1939.

The strain used in the present study was GWM *6412*. "Hülle" cells are considerably more abundant in this species than in *E. quadrilineata*, and the ascocarps are often clumped in habit. The ascocarp matures

somewhat more slowly than in some species; consequently, crozier formation is readily demonstrable and ascal morphology easily observed. The ascocarps of an individual clump are usually in different stages of development so that one can often see several stages in a single paraffin section.

The genus *Emericella* seems quite distinct on the basis of the structure of the mature ascocarp, the occurrence of "hülle" cells, the development of the ascocarp, and the frilled nature of the spores. It would seem that some support would be given to the maintenance of the imperfect genus *Sterigmatocystis* Cramer also, inasmuch as the perfect stages are so distinctly separated from *Eurotium* and *Sartorya*, the perfect stages of the "single-ranked-sterigmate" Aspergilli.

Talaromyces gen. nov.

Peritheciis minutis, globosis vel subglobosis, superficialibus, sine ostiolo, plerumque sulfureis, laxe textis; ascis aut singulis aut catenatis, ovatis vel globosis, octosporis, cito diffluentibus; ascosporis hyalinis, continuis; fructificatione conidica generi *Penicillio* Link ex Fries ascribenda.

Etym. τάλαρος (basket) + μύκης.

Perithecia small (100–500 μ), globose to subglobose, superficial, nonostiolate, mostly yellow, discrete but with a tendency toward confluence in some species, of indeterminate growth; perithecial wall composed of interwoven hyphae, consistency various, from very loose texture to more or less compressed; ascocarpic initials various; asci numerous, ovate to globose, predominantly borne in short chains, more rarely singly and then formed by croziers, irregularly disposed throughout the ascocarp and deliquescing at maturity; ascospores mostly spiny, more rarely smooth or girdled, continuous, hyaline or nearly so; conidial stage *Penicillium* Link ex Fries.

Type species: *Talaromyces vermiculatus* (Dang.) C. R. Benj.

Talaromyces luteus (Zukal) comb. nov. FIG. 17

Penicillium luteum Zukal (Sitz.-Ber. Akad. Wien **98**: 561. 1889.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. The strain used in the present study was NRRL 2235. The description of the ascocarpic stage is discussed by Raper and Thom and by Emmons (**1935**) under the imperfect stage name, *Penicillium luteum*.

Talaromyces luteus appears to be one of the simpler members of the genus. Developmentally and anatomically it appears to be almost as

closely related to the genus *Arachniotus* of the Gymnoascaceae as to some of the other members of *Talaromyces*. Placement of the species in *Arachniotus*, however, would necessitate removal of other species, such as *Talaromyces striatus*, to which it is obviously closely related, to that genus also. This would seem unwarranted. As a matter of fact, the whole genus *Talaromyces* would seem to be composed of a series of forms intermediate between the lower members of the Gymno-ascaceae and the Eurotiaceae. Inclusion of *Talaromyces* in the Eurotia-ceae would indicate that the two families were derived from ancestors at about the same level of development. Or to state it somewhat differ-ently, it would, in accordance with the modern taxonomic viewpoint, afford some phylogenetic depth to the family Eurotiaceae.

Talaromyces avellaneus (Thom & Turesson) comb. nov. Fig. 7

Penicillium avellaneum Thom & Turesson (Mycologia **7**: 284. 1915.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Both the ascocarpic and conidial stages are described by Raper and Thom (**1949**). Subcultures of NRRL *1938*, representing the type strain, were used in the present study.

The ascocarpic initials of this strain appeared to be relatively undif-ferentiated, much as described by Emmons (**1935**) for *T. wortmannii*. The mature ascocarp also is quite simple, consisting of a mass of fertile tissue surrounded by hyphae undifferentiated into any definite peridium or wall. When grown on Czapek's agar the wall is indistinguishable from the sterile mass of colony hyphae. The asci are quite large, some-times reaching a length of 20 μ, are formed singly from the penultimate cells of crozier formations of the ascogenous hyphae, are relatively thick-walled, and contain eight large ascospores. *T. avellaneus* would seem to be more closely related to *T. luteus* than perhaps to any other species of the genus because of the texture of the ascocarp wall and the forma-tion of croziers.

Talaromyces striatus (Raper & Fennell) comb. nov. Figs. 6, 20

Penicillium striatum Raper & Fennell (Mycologia **40**: 521. 1948.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Both stages were described by the authors. Subcultures of the type strain NRRL *717* were used for the present study.

The ascocarp appears to be initiated by an ascogonial coil. No structure was observed which could have been considered an antheridium, but further developmental study will be necessary before any positive

statement can be made regarding its absence. The mature ascocarp wall is composed of the interwoven hyphae characteristic of the genus and seemed somewhat more differentiated than in *T. wortmannii, T. luteus* and *T. avellaneus*. The asci are not formed in chains such as are found in the majority of the species of the genus, nor do they arise as lateral or terminal branches of the ascogenous hyphae after the fashion of *Carpenteles*. Rather they are formed from the penultimate cells of croziers after the fashion of *T. luteus* and *T. avellaneus*. However, the ascogenous hyphae become emptied of cytoplasm and collapse, so that crozier formation is difficult to demonstrate except in young ascocarps. The species is thought to be most closely related to *T. luteus* and *T. avellaneus*, the three forms making up a series somewhat divergent from the other species of the genus.

Talaromyces wortmannii (Klöcker) comb. nov. FIG. 16

Penicillium wortmanni Klöcker (Compt. Rend. Lab. Carlsberg **6**: 100. 1903.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Descriptions of both ascocarpic and conidial stages are given by Raper and Thom (**1949**), and the development of the ascocarp was observed by Emmons (**1935**). Subcultures of NRRL *1017* were used in this study. The species appears to be the most simple of those forms in which the asci are borne in chains and apparently are not formed by croziers, differing in this respect from the preceding species of the genus.

Talaromyces spiculisporus (Lehman) comb. nov.

Penicillium spiculisporum Lehman (Mycologia **12**: 268. 1920.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Good descriptions of both stages are given by Raper and Thom (**1949**). The development of the ascocarp was described by Emmons (**1935**) and need not be repeated here. The strain NRRL *1026* was used in the present study.

Talaromyces rotundus (Raper & Fennell) comb. nov. FIGS. 5, 18, 19

Penicillium rotundum Raper & Fennell (Mycologia **40**: 518. 1948.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Subcultures of the authors' type strain, NRRL *2107*, were used in the present study, which confirmed their observations on the ascocarp and its initiation. The perithecial initials are

very similar to those found in *T. wortmannii*, so well described by Emmons (**1935**).

Talaromyces bacillosporus (Swift) comb. nov.

Penicillium bacillosporum Swift (Bull. Torrey Bot. Club **59**: 221. 1932.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Both ascocarpic and conidial stages are included in the description by Raper and Thom (**1949**). The strain used by them, NRRL *1025* representing type material, was used for the present study, which confirmed only their observations on the mature ascocarp since no developmental study is included here. The wall of the mature ascocarp, although made up of interwoven hyphae as in the other species of the genus, was somewhat more compact than in species such as *T. wortmannii* and *T. luteus*. It would seem that the level of development of *T. bacillosporus* is about that of *T. striatus* with respect to wall structure.

Talaromyces vermiculatus (Dang.) comb. nov.

Penicillium vermiculatum Dangeard (Le Botaniste **10**: 123. 1927.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. Raper and Thom (**1949**), Emmons (**1935**), and Dangeard (**1907**) have all included discussions of the development and morphology of the ascocarp. Two strains were used in the present study, NRRL *2098* and SUI *1725*, with confirmatory results.

Talaromyces stipitatus (Thom) comb. nov.

Penicillium stipitatum Thom (Emmons, Mycologia **27**: 138. 1935.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. The strains used in the present study were NRRL *2105* and GWM *6372*. The latter was isolated from bark of *Pinus virginiana* collected in Clark Co., Indiana, 1945. Good discussions of the ascocarpic stage are presented both by Emmons (**1935**) and Raper and Thom (**1949**) under the name *P. stipitatum*. Discussion here would seem redundant.

Talaromyces helicus (Raper & Fennell) comb. nov.

Penicillium helicum Raper & Fennell (Mycologia **40**: 515. 1948.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage. The ascocarpic stage was included in the original

description, which was based on NRRL *2106*, the same strain used in the present study.   Developmental studies of the species are not included in the present study, but observations made on both fresh material and paraffin sections tend to confirm the observations reported by Raper and Fennell (**1948**).

CARPENTELES Langeron, Compt. Rend. Soc. Biol. Paris **87**: 344.   1922.

> Type species: *Carpenteles asperum* Shear, Mycologia **26**: 107.   1934.
> Conidial stage: *Penicillium* Link ex Fries.

CARPENTELES JAVANICUM (van Beyma) Shear, Mycologia **26**: 107. 1934.   FIG. 21

The imperfect stage of this species is *Penicillium javanicum* van Beyma as described by Raper and Thom (**1949**), who included the ascocarpic stage along with the description of the conidial stage.   Subcultures of NRRL *707*, the type strain, were used in the present study. Discussions of both developmental and mature morphology of the ascocarps of members of *Carpenteles* have been presented by Dodge (**1933**), Emmons (**1935**), and Raper and Thom (**1949**).   It would seem that species included in the genus are very closely related both on the basis of development and on the basis of the morphology of the mature ascocarp.

CARPENTELES ASPERUM Shear, Mycologia **26**: 107.   1934

The imperfect stage of this species is the form described by Raper and Thom (**1949**) as *Penicillium asperum* (Shear).   Subcultures of NRRL *715*, representing type material, were used in the present study.
   C. asperum Shear, whether or not it represents the material studied by Brefeld (**1874**) as *Penicillium glaucum* Lk. which was designated as type by Langeron (**1922**), is considered the type species of the genus. Its development and mature morphology are, as a matter of fact, quite representative of the whole series of forms belonging in the genus.

Carpenteles levitum (Raper & Fennell) comb. nov.   FIG. 8

Penicillium levitum Raper & Fennell (Mycologia **40**: 511.   1948.) is a synonym with respect to the ascocarpic form, but the name is valid for the conidial stage.   Both stages were described by the authors under the imperfect stage name.   The present study was based on SUI *1724*, a strain isolated from soil collected near Iowa City, Iowa, by R. Bandoni.

As in other members of the genus, in *C. levitum* the ascocarp is initiated by a plexus of cells in the crotch of a "tree-like" network of hyphae, as illustrated on p. 55 by Raper and Thom (**1949**). The nature of the mature ascocarp wall deviates somewhat from the sclerotioid type so characteristic of the genus, but the arrangement and origin of the asci as terminal and lateral buds from the ascogenous hyphae is as reported by Emmons (**1935**) for the preponderance of the species. The latter character plus the mode of origin of the ascocarp would seem to relate the species sufficiently to others to warrant the inclusion of *C. levitum* in this genus.

SUMMARY

A morphological study of the ascocarps of 29 strains of *Aspergillus* and *Penicillium* indicates that these forms comprise five genera of the Eurotiaceae. The perfect stages of *Aspergillus* are included in the genera *Eurotium, Sartorya*, and *Emericella*. Those of *Penicillium* are included in *Carpenteles* and *Talaromyces*, the latter a new genus erected for the forms with ascocarpic walls of interwoven hyphae. Species which had not previously been assigned to an ascomycetous genus are here so assigned.

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THE HOST SPECIALIZATION OF ERYSIPHE CICHORACEARUM FROM ZINNIA, PHLOX AND CUCURBITS [1]

John A. Schmitt, Jr.

Erysiphe cichoracearum DC. ex Mérat, one of the more ubiquitous powdery mildews, has been widely used in inoculation experiments to determine host range. The literature on the earlier inoculation experiments with this mildew is reviewed by Blumer (**1933**). From the earlier experiments it was concluded by mycologists in general that this mildew exhibited a strong specialization, at least at the family level. Blumer strongly emphasized host specialization in his species concept and thus was able to separate ten species from *Erysiphe cichoracearum* in the broad sense. For many of these species he claimed correlated morphological differences sufficient for specific recognition.

Hammarlund (**1945**) described *Erysiphe polyphaga*, differing morphologically from *E. cichoracearum* by its 4-spored, as well as 2- and 3-spored asci. The principal diagnostic feature for *E. polyphaga*, however, was its host specialization. Hammarlund reported successful cross-inoculations of his mildew from *Cucumis sativus* to 62 of 99 species in the Crassulaceae, Scrophulariaceae, Primulaceae, Saxifragaceae, Begoniaceae, Solanaceae, Cucurbitaceae, Labiatae, Compositae, Verbenaceae and Euphorbiaceae. Blumer (**1950, 1952**), using a cucurbit mildew he considered to be *E. polyphaga*, reported successful infection on members of the Crassulaceae, Scrophulariaceae, Labiatae, Ericaceae, Vitaceae, Boraginaceae, Gesneriaceae and Compositae. Thus, there was demonstrated a wide host range for a mildew difficult to distinguish morphologically from *Erysiphe cichoracearum*.

The present study was initiated to investigate the host specialization of accessions of *Erysiphe cicroracearum* DC. ex Mérat from zinnia (*Zinnia elegans*), sunflower (*Helianthus annuus*) and *Inula helenium* of the Compositae; from perennial cultivated phlox (*Phlox paniculata, P. maculata*, or their hybrids) of the Polemoniaceae; from squash (*Cucurbita pepo*) of the Cucurbitaceae; and *Cerinthe major* of the Boraginaceae.

[1] This represents a portion of the research presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Michigan.

MATERIALS AND METHODS

The original cultures of the mildew on zinnia, sunflower, phlox and squash were obtained from the field in the vicinity of Ann Arbor, Michigan, while those on *Inula helenium* and *Cerinthe major* developed as a result of infection following inoculations from one of the field-grown mildews. Later, an additional accession of the mildew on *Inula* and *Cerinthe* was obtained from the field. Each original accession was sub-cultured at least three times to purify the mildew before being used as an inoculum source.

The majority of the inoculations utilized a technique of dusting conidia from the inoculum source onto the leaves of the experimental plants, after first spraying the plants with distilled water. This technique proved as successful as that of atomizing a conidial suspension onto the leaves of the plants.

Plants of the species to be inoculated were grown from seeds. Seedlings were inoculated usually at the two- and four-leaf stage, although occasionally a more mature plant was inoculated to check for possible differences in susceptibility due to the age of the plant. In each experiment plants of the species from which the conidia were taken were inoculated along with the experimental plants to check the viability of the conidia. Controls were run with each experiment.

Inoculated plants were incubated at 60–70° F, and were subjected to supplementary illumination (during winter months) from a 300 watt incandescent bulb to increase day length to about fifteen hours. The additional light tended to produce more vigorous plants, which according to Reed (1915) will effect better development of the fungus. No attempt was made to control the relative humidity in the greenhouse rooms since this seems to be of little importance in the development of mildews, according to the results of Duvdevani *et al.* (1946) for the cucurbit mildew.

The inoculation chambers used for the experiments were those described by Mains (1924). In each experiment the chambers were filled with experimental plants in a room other than that in which the inoculum sources were maintained. The control chambers were not opened during the course of the experiments. After inoculation of the experimental plants, the chambers were closed for a 24–48-hour incubation period, at the end of which they were opened partially to allow for circulation of air. Three or four days after inoculation the experimental plants were removed from the chambers to the greenhouse benches. Observations were made usually on the fifth day after inoculation and

were checked on the seventh, eighth or ninth day. The fifth day was chosen for the initial observation since previous workers reporting on this mildew agree that infection will become evident by the fourth to sixth day after inoculation. The second observation was found necessary to determine the correct reaction type.

The results of the inoculations were graded on the following system of reaction types:

  0—no infection evident macroscopically;

  I—sparse mycelial development with few or no conidia produced;

  II—abundant mycelial development with no or only a few conidia produced;

III—mycelium and conidia developed abundantly.

Many of the tests with the different species of hosts were repeated several times throughout each of the three years during which this investigation was carried out. In each experiment several to many plants of the different species were inoculated.

<center>RESULTS</center>

Data obtained from the inoculation experiments are presented in TABLE I.

A. RESULTS WITH ERYSIPHE CICHORACEARUM FROM ZINNIA ELEGANS

FROM TABLE I it can be seen that a much more widespread range of species susceptible to this mildew occurs in the Compositae than in any of the other families. Schmitt (**1954**) only recently added *Zinnia pauciflora, Z. verticillata* and *Salpiglossis sinuata* to the list of species susceptible to *Erysiphe cichoracearum*.

Of particular note is the fact that in the following otherwise susceptible species resistant plants were found: *Xanthium chinense*, 3 of 12 plants; *Mikania scandens*, 4 of 22 plants; *Lactuca perennis*, 4 of 18 plants; *Crepis foetida*, 10 of 32 plants; and *Salpiglossis sinuata*, 1 of 13 plants.

The mildew from the infections on *Xanthium chinense*, *X. spinosum*, *Arctium minus*, *A. nemorosum*, *Lactuca perennis*, *Felicia amelloides*, *Cosmos* sp., *Zinnia pauciflora* and *Z. verticillata* was successfully transferred back to *Zinnia elegans*. The mildew obtained from *Arctium minus* was also successfully transferred to *Helianthus annuus* and *Cerinthe major*.

B. Results with E. cichoracearum from Inula helenium

This mildew was inoculated onto the 22 species shown in Table I. Species infected were *Inula helenium, Zinnia elegans, Helianthus annuus* and *Cerinthe major,* which were also susceptible to the mildew from *Zinnia elegans.*

C. Results with E. cichoracearum from Cerinthe major

The species susceptible to this mildew can be seen from Table I. This mildew was found to have almost the same host range as the mildew from *Zinnia elegans.* The one exception to common susceptibility by both mildews is *Lactuca perennis,* which was susceptible to the zinnia mildew, but on which the mildew from *C. major* failed to develop.

With this, as with the two previous mildews, there was some variability in the degree of susceptibility of certain hosts to the mildew. For example, 2 of 12 plants of *Helianthus annuus* showed no infection.

D. Results with E. cichoracearum from Helianthus annuus

From the table it can be seen that the species susceptible to this sunflower mildew are the same as those infected by the mildew from zinnia, with three exceptions. Inoculations of this sunflower mildew onto *Hieracium alpinum* (20 plants), *H. prenanthoides* (16 plants) and *Carlina acaulis* (1 plant) failed to produce infection.

As mentioned previously, resistant plants were found in species otherwise susceptible to this mildew: *Inula helenium,* 1 of 8 plants; and *Lactuca perennis,* 7 of 20 plants.

During the winter of 1951–52 these tests were carried out with an accession obtained after infection of sunflower with conidia of the zinnia mildew. Another accession was obtained from the field during the summer of 1952, which was used for the tests during 1952–53. Only the second accession was tested on *Cerinthe major* and *Inula helenium.* The field accession was identified as *Erysiphe cichoracearum* by perithecial characteristics.

E. Results with E. cichoracearum from perennial phlox

The mildew from the cultivated perennial phlox was found to have a very narrow specialization. Of the 34 species of hosts for *Erysiphe cichoracearum* which were inoculated, infection occurred only on *Phlox drummondii* and the cultivated perennial phlox. In the case of *P. drummondii* 1 plant of 15 did not become infected by this mildew. Mains

Table I

RESULTS OF INOCULATION EXPERIMENTS WITH ERYSIPHE CICHORACEARUM
FROM VARIOUS SOURCES

Host plants inoculated:	Reactions of the hosts to the mildew from:					
	Zinnia elegans	Inula helenium	Cerinthe major	Helianthus annuus	Phlox (cult.)	Cucurbita pepo
Compositae:						
Zinnia elegans	III (38)[1]	III (3)	III (4)	III (3)	0 (8)	0 (6)
	II (18)	II (2)	II (7)	I (3)		
Z. pauciflora	III (20)	—[2]	II (18)	II (32)	—	—
Z. verticillata	III (12)	—	II (5)	II (26)	—	—
Prenanthes purpurea	0 (8)	—	—	0 (5)	0 (3)	—
Sonchus arvensis	0 (9)	—	—	—	0 (4)	0 (3)
Tanacetum vulgare	0 (3)	—	—	—	—	0 (1)
Helianthus annuus	III (5)	III (2)	II (10)	III (9)	0 (8)	0 (8)
	II (13)	II (2)	0 (2)			
Arctium minus	II (13)	—	II (5)	II (4)	0 (4)	0 (8)
A. nemorosum	II (15)	—	II (5)	II (6)	—	—
Taraxacum officinalis	0 (3)	—	—	—	0 (2)	0 (3)
Centaurea cyanus	0 (7)	0 (4)	—	0 (4)	—	—
C. scabiosa	0 (4)	—	—	—	—	—
C. dealbata	0 (4)	—	—	—	—	—
C. jacea	0 (11)	—	—	—	0 (5)	—
C. macrophylla	0 (2)	—	—	—	0 (4)	—
C. melitensis	0 (33)	—	0 (18)	0 (12)	—	—
Xanthium chinense	III (9)	—	—	—	—	0 (2)
	0 (3)					
X. strumarium	II (3)	—	—	—	—	—
Xanthium spinosum	III (1)	—	—	—	0 (4)	0 (2)
Mikania scandens	III (1)	—	—	—	—	—
	II (17)					
	0 (4)					
Cichorium intybus	0 (18)	0 (18)	0 (7)	0 (5)	—	—
Carduus acanthoides	0 (10)	—	—	—	0 (3)	—
C. nutans	0 (6)	—	—	—	0 (4)	—
Rudbeckia hirta	0 (5)	—	—	—	—	—
Calendula officinalis	0 (12)	0 (6)	0 (8)	0 (5)	0 (4)	0 (2)
Lapsana communis	0 (6)	—	—	—	0 (4)	—
Eupatorium cannabinum	0 (10)	—	—	—	0 (5)	0 (2)
Hieracium alpinum	III (23)	—	III (24)	0 (20)	—	—
H. amplexicaule	0 (16)	—	0 (10)	0 (10)	—	—
H. murorum	0 (5)	—	0 (3)	—	0 (4)	0 (2)
H. pilosella	0 (16)	0 (5)	0 (9)	0 (7)	—	—
H. prenanthoides	III (19)	—	III (18)	0 (16)	—	—
H. silvaticum	0 (12)	—	—	0 (8)	—	—
Inula helenium	III (8)	III (3)	III (1)	I (7)	0 (3)	—
	II (12)		I (3)	0 (1)		
Carlina acaulis	II (3)	—	I (3)	0 (1)	—	—
Achillea millefolium	0 (16)	—	0 (4)	—	0 (3)	0 (5)
Dahlia sp. (annual)	0 (1)	—	—	—	0 (2)	—
Cirsium vulgare	0 (10)	—	—	—	—	—
C. arvense	0 (2)	—	—	—	0 (3)	0 (3)
C. canum	0 (4)	—	—	—	—	—
Artemisia absinthium	0 (11)	0 (5)	0 (6)	0 (5)	—	—

[1] The number in parentheses is the number of plants giving the reaction.
[2] The dash indicates that no test was carried out with that combination of host and mildew source.

TABLE I—*Continued*

Host plants inoculated:	Reactions of the hosts to the mildew from:					
	Zinnia elegans	*Inula helenium*	*Cerinthe major*	*Helianthus annuus*	*Phlox* (cult.)	*Cucurbita pepo*
Compositae (*Continued*):						
A. vulgaris	0 (7)	0 (6)	0 (7)	0 (6)	—	—
Lactuca perennis	II (14)	0 (7)	0 (21)	II (16)	—	—
	0 (4)			0 (7)		
Cosmos sp.	III (3)	—	II (5)	III (3)	—	—
	II (10)			II (10)		
Helichrysum bracteatum	0 (10)	0 (5)	0 (8)	—	—	—
Aster alpinus	0 (16)	—	—	0 (5)	—	—
Scorzonera hispanica	III (8)	—	II (8)	II (8)	—	—
	II (6)					
Crepis foetida	II (22)	—	II (22)	II (4)	—	—
Felicia amelloides	II (32)	—	II (20)	—	—	—
Cucurbitaceae:						
Cucurbita pepo (CP 434 Al)	0 (4)	—	—	—	0 (6)	III (12)
(25-5XX)	0 (4)	—	—	—	0 (5)	III (4)
(Zuccini × White Disk)	—	—	—	—	—	III (3)
(Conn. Field Pumpkin)	0 (6)	0 (5)	0 (2)	0 (5)	—	II (1)
C. maxima	0 (5)	0 (2)	0 (4)	0 (6)	—	II (3)
C. melanosperma	0 (3)	—	0 (2)	0 (5)	—	—
Echinocystis lobata	0 (3)	—	—	—	—	II (3)
Sicyos angulatus	—	—	—	—	—	II (3)
Cyclanthera pedata	0 (4)	0 (2)	0 (3)	0 (4)	—	—
Momordica charanthia	0 (4)	0 (4)	0 (5)	0 (2)	—	0 (4)
Citrullus calycanthidis	0 (3)	—	0 (6)	0 (4)	—	—
Bryonia dioica	0 (4)	—	0 (4)	0 (7)	—	0 (6)
Solanaceae:						
Hyoscyamus niger	0 (15)	0 (5)	0 (15)	0 (15)	—	—
Salpiglossis sinuata	II (15)	—	I (5)	I (5)	—	—
	0 (1)					
Nicotiana tabacum	0 (19)	—	0 (10)	0 (10)	—	—
Boraginaceae:						
Symphytum officinale	0 (17)	—	0 (25)	0 (10)	0 (2)	0 (1)
Anchusa italica	0 (2)	—	—	—	0 (2)	0 (1)
Cerinthe major	III (5)	II (4)	III (11)	III (2)	0 (3)	—
	II (18)	I (1)	II (6)	II (3)		
C. minor	0 (5)	—	0 (10)	0 (9)	—	—
Cynoglossum officinale	0 (5)	—	0 (5)	—	—	—
Plantaginaceae:						
Plantago alpina	0 (13)	—	0 (5)	0 (10)	—	—
P. coronopus	0 (16)	—	—	0 (6)	—	—
P. lanceolata	0 (12)	—	0 (11)	0 (7)	0 (3)	—
P. major	0 (4)	—	—	0 (16)	0 (3)	0 (4)
Plantago maritima	0 (11)	0 (8)	0 (8)	0 (4)	0 (3)	—
P. media	0 (10)	0 (6)	0 (7)	0 (9)	—	—
P. rugelii	0 (12)	—	—	0 (13)	0 (4)	0 (9)
Polemoniaceae:						
Phlox sp. (Cult. perennial)	—	—	—	—	III (4)	—
P. drummondii	0 (15)	—	0 (5)	0 (5)	III (14)	0 (4)
					0 (1)	
Cobaea scandens	0 (2)	—	—	—	0 (4)	0 (4)
Polemonium van-bruntiae	0 (2)	—	—	—	0 (3)	—
P. flavum	0 (14)	0 (4)	0 (6)	0 (4)	—	—
P. pauciflorum	0 (8)	—	—	0 (3)	—	—
Polemonium carneum	0 (15)	—	—	—	—	—

TABLE I—*Continued*

Host plants inoculated:	Reactions of the hosts to the mildew from:					
	Zinnia elegans	*Inula helenium*	*Cerinthe major*	*Helianthus annuus*	*Phlox* (cult.)	*Cucurbita pepo*
Polemoniaceae (*Continued*):						
Gilia rubra	0 (10)	—	0 (4)	0 (4)	—	—
Navarettia squarrosa	0 (10)	—	0 (8)	0 (7)	—	—
Collomia abrotanifolia	0 (8)	—	—	—	—	—
Labiatae:						
Teucrium chamaedrys	0 (2)	—	—	—	—	0 (1)
Prunella vulgaris	0 (4)	—	—	—	—	0 (4)
Salvia glutinosa	0 (1)	—	—	—	0 (3)	—
Stachys palustris	0 (5)	—	—	—	0 (4)	0 (3)
Scrophulariaceae:						
Antirrhinum majus	0 (22)	—	0 (6)	0 (4)	—	—
Verbascum nigrum	0 (14)	0 (5)	0 (8)	0 (6)	—	—
V. thapsus	0 (17)	—	0 (4)	0 (9)	—	—
Violaceae:						
Viola sp. (cult.)	0 (2)	—	—	—	—	0 (2)
Cruciferae:						
Cardamine impatiens	0 (16)	—	0 (10)	0 (5)	—	—
Rubiaceae:						
Galium aparine	0 (30)	—	0 (12)	0 (14)	—	—

(**1942**) has found that both the perennial phlox and *P. drummondii* vary in their reaction to the mildew and has reported resistant selections.

F. RESULTS WITH E. CICHORACEARUM FROM CUCURBITS

The results of the experiments with the two accessions of the cucurbit mildew indicate a restriction to the Cucurbitaceae. TABLE I shows that inoculations of this mildew onto species in families other than the Cucurbitaceae failed to cause infection. There is, however, a specialization to certain genera and species within this host family, since plants of *Momordica charanthia* and *Bryonia dioica* were not infected by a mildew obtained from *Cucumis sativus*.

DISCUSSION

The three forms of *Erysiphe cichoracearum sensu* Salmon demonstrated by this study, the zinnia, phlox, and curbit mildews, are distinct by virtue of their specialization. The mildews on *Zinnia elegans, Inula helenium, Helianthus annuus* and *Cerinthe major* are considered to be one form because of the similarity in host range, as demonstrated by the inoculation experiments herein reported. The phlox mildew has a very apparent limited host spectrum. The form on cucurbits is intermediate in specialization between the other two forms.

Comparisons of the host specialization for the mildews of the present study with previously recognized forms or biologic species present difficulties. While it is desirable that the same species of hosts be used in all studies of host specialization it is difficult to obtain seeds of all species of hosts used by all investigators. Consequently, the mildews have been tested on as many as possible of the species listed by Salmon (**1900**) as hosts for *Erysiphe cichoracearum*. Comparisons of the specialization of the zinnia, phlox and cucurbit mildews of the present study with that of two special forms of *E. cichoracearum sensu* Blumer (**1933**) follow. The comparisons are made to determine to which of these taxa designated by Blumer the present three forms might belong.

Erysiphe cichoracearum as treated by Blumer (**1933**) is reported on a number of species susceptible to the zinnia mildew. *Scorzonera hispanica* is cited as the only susceptible species for Blumer's *E. cichoracearum* f. sp. *scorzonerae*, which was established by Blumer as a result of Bouwens' experiments (**1927**). In Bouwens' experiments none of the other species used in the present study was inoculated, so a direct comparison is not possible. In view of the results on *Scorzonera hispanica* the zinnia mildew may be the same as the form she studied. However, studies to test the European mildew on *S. hispanica* on the other species susceptible to the zinnia mildew are necessary before this can be established definitely.

Blumer established *Erysiphe cichoracearum* f. sp. *euhieracii* as a result of experiments using a mildew from *Hieracium murorum* (**1922b**). It infected 12 other species of the subgenus *Euhieracium*, one of which was *H. prenanthoides*. *Hieracium prenanthoides* was found to be susceptible to the zinnia mildew in the present investigation, whereas *H. murorum* and *H. silvaticum*, which are hosts for f. sp. *euhieracii* of Blumer, were not infected by the zinnia mildew. *Hieracium prenanthoides*, therefore, is a favorable host for two different forms. No other form of *Erysiphe cichoracearum sensu* Blumer has hosts in common with the zinnia mildew.

Blumer lists as hosts for *Erysiphe cichoracearum* 38 genera of the Compositae, plus 3 species of the Violaceae and 1 species each of the Polemoniaceae (*Polemonium van-bruntiae*) and the Solanaceae (*Nicotiana tabacum*). Only a few of the species listed by Blumer were previously used in studies of host specialization. He included *Carlina acaulis, Hieracium prenanthoides, Inula helenium, Scorzonera hispanica, Xanthium strumarium*, and *X. spinosum*, which are susceptible to the zinnia mildew. The zinnia mildew did not infect *Polemonium van-*

bruntiae, Nicotiana tabacum and *Viola* sp., but it did infect the borage, *Cerinthe major,* and the solanaceous species *Salpiglossis sinuata.*

Erysiphe depressa (Wallr.) Schlecht., as recognized by Blumer (**1933**), has as hosts species of *Arctium* in common with the zinnia mildew. Blumer states that *E. depressa* from *Arctium minus* infected *A. lappa, A. palladium, A. nemorosum,* and *A. tomentosum.* Both *A. minus* and *A. nemorosum* are hosts for the zinnia mildew. Blumer did not inoculate the other species which are susceptible to the zinnia mildew. Bouwens was unable to secure infection of *Scorzonera hispanica* with a mildew from *A. minus.* Since both *S. hispanica* and *A. minus* were susceptible to the zinnia mildew at least two forms have *A. minus* as a host.

Blumer (**1933**) recognized *Erysiphe montagnei* Lév. as being restricted to 9 species of *Cirsium. Cirsium arvense,* a host for *E. montagnei,* was not infected by the zinnia mildew, but inasmuch as only 2 plants were inoculated, the results are not conclusive. Since *E. montagnei* was not tested on species other than those in *Cirsium,* the zinnia mildew may conceivably be this species recognized by Blumer.

Erysiphe artemisiae (Wallr.) Grev., as recognized by Blumer (**1933**), is restricted to species of *Artemisia* of the Compositae. The zinnia mildew was tested on *Artemisia absinthium* and *A. vulgaris,* both of which are listed as hosts for *E. artemisiae* by Blumer; they were not infected by the zinnia mildew.

Erysiphe horridula Lév. was retained by Blumer, partly on the basis of a supposed restriction to the Boraginaceae from the data of his experiments (**1922a**) and partly because of the often 3-spored asci produced by the fungus on some boraginaceous hosts. In his experiments, however, he did not inoculate any species other than those in the Boraginaceae. Blumer cited *Cerinthe major* as a common host for all forms of the borage mildew which he investigated. In the present experiments *C. major* was always infected by the zinnia mildew, while *Symphytum officinale, Anchusa italica, Cynoglossum officinale* and *Cerinthe minor* (listed by Blumer as hosts for *E. horridula*) were not infected.

Salmon (**1900**), on the other hand, listed *Cerinthe major* as a host for *Erysiphe cichoracearum.* No perithecia of the mildew developed on *C. major* during the experimental period of the present study, so specific determination of the mildew was not possible. Circumstantial evidence, however, tended to establish the mildew as *E. cichoracearum.* The original culture of this mildew was obtained from the field in the summer of 1952. The plants were growing next to zinnias which were heavily

infected with *E. cichoracearum*. Because of this proximity to plants heavily infected with *E. cichoracearum*, and because the host relationships of the mildew on *Cerinthe major* are similar to those of the mildew on zinnia, the mildew on *C. major* appears to be *E. cichoracearum*. The original accession of the mildew on *C. major*, derived from a field infection of this host, reacted identically with an accession obtained after infection of *C. major* with conidia of the mildew on zinnia.

Erysiphe polyphaga is reported on several species which are susceptible to the zinnia mildew. From the cultural studies of Hammarlund (**1945**) and Blumer (**1950, 1952**) *E. polyphaga* occurs on 85 species in 38 genera of 15 families of flowering plants. This is a considerably greater range of hosts than that of the zinnia mildew, which infected 19 species in 13 genera of composites and 1 species each of the Boraginaceae and Solanaceae. *Erysiphe polyphaga* and the zinnia mildew have been shown to infect *Helianthus annuus* and *Scorzonera hispanica* of the Compositae. The following hosts for *E. polyphaga* were not infected by the zinnia mildew: *Viola* sp. (probably *V. tricolor maxima* hort.), *Nicotiana tabacum*, *Dahlia* sp. (probably *D. variabilis*), and *Antirrhinum majus*. The zinnia mildew has a host range limited primarily to the Compositae, but including species from rather distantly related families.

No special forms or biologic species have been established previously, as a result of inoculation experiments, on species of *Xanthium, Mikania, Inula, Carlina, Lactuca, Cosmos, Felicia,* or *Salpiglossis,* which have been found to be susceptible to the zinnia mildew.

The results of the inoculation experiments using conidia of the phlox mildew show that it is restricted to *Phlox drummondii* and to the cultivated perennial phlox. Salmon (**1900**) lists only *Phlox divaricata, P. drummondii,* and *P. paniculata* from the Polemoniaceae, as hosts for *Erysiphe cichoracearum*. Species of the Polemoniaceae have not been used in other studies of the host specialization of the powdery mildews. Of the Polymoniaceae, Blumer (**1933**) cites only *Polemonium vanbruntiae (P. caeruleum)* as a host for *Erysiphe cichoracearum*. This species of host was included by Blumer because of morphological characteristics of the mildew on this host, not as the result of inoculation experiments. *Polemonium van-bruntiae* was never infected by the phlox mildew during the present experiments.

Neither Hammarlund nor Blumer inoculated *Erysiphe polyphaga* onto species of *Phlox*. The phlox mildew failed to infect *Helianthus annuus, Dahlia* sp. (probably *D. variabilis*) and *Viola* sp. (probably *V. tricolor maxima* hort.), which are hosts for *E. polyphaga*.

Several different mildews have been reported on the Cucurbitaceae, namely *Erysiphe cichoracearum* (Salmon, **1900**; Reed, **1907**, **1908**; Jørstad, **1925**) ; *E. polygoni* (Salmon, **1900**) ; *E. polyphaga* (Hammarlund, **1945**; Blumer, **1950**, **1952**) ; *Sphaerotheca fuliginea* (Blumer, **1933**) ; *S. "Castagnei"* (Salmon, **1900**) ; and *Oidium* sp. (various authors). Since no perithecia developed on members of the Cucurbitaceae during the present study, the true identity of the fungi remains in question. Some of the collections reported by Salmon (**1905**: 249) and Reed (**1907**: 533) bore perithecia of *Erysiphe cichoracearum*. Bouwens (**1924**: 22) decided the mildew on cucurbits was *E. cichoracearum*, basing her conclusion on conidial measurements.

The results of the author's cucurbit mildew study and of that by Miller and Barrett (**1931**) indicate a specialization to the Cucurbitaceae. In the present investigation *Cucurbita pepo, C. maxima, Echinocystis lobata* and *Sicyos angulatus* were susceptible, whereas *Momordica charanthia* and *Bryonia dioica*, as well as species from other families, were not infected. Reed (**1907**, **1908**) obtained infection on *Momordica charanthia* with a mildew from *Cucurbita maxima*. He also reported infection of *Helianthus annuus* and *Plantago rugelii* by a mildew from *C. maxima*. Reed stated that the mildews that developed on *H. annuus* and *P. rugelii* were successfully transferred back to squash plants. Although infections occurring on control plants throw some doubt on Reed's results, the successful reciprocal infections seem to authenticate his results on *H. annuus* and *P. rugelii*. Miller (**1938**) could not secure infection of sunflower with the cantaloupe mildew, nor was the reciprocal successful. The cucurbit mildew used in the present study did not infect either *H. annuus* or *P. rugelii*. It therefore appears to be distinct from the mildew studied by Reed.

Only *Helianthus annuus* and *Viola* sp., among the species of hosts for *Erysiphe polyphaga*, were inoculated with the cucurbit mildew used in the experiments reported herein. Neither species became infected. The host range of the writer's cucurbit mildew is much narrower than that of *E. polyphaga*. The cucurbit mildew under investigation was tested on a rather wide range of host families and it would seem that any tendency toward a wide host range would have become apparent in the results of the experiments.

In the past many investigators have inoculated hosts from only one family or in some instances from only one genus. From the results of these inoculations they have then established the host range for the particular mildew. Such experiments decidedly limit the range of hosts that can be demonstrated. The following species recognized by Blumer

(1933) were based on such limited-range inoculation experiments:
Erysiphe fischeri (Blumer, 1922b); *E. salviae* (Blumer, 1933; Laibach,
1930); *E. lamprocarpa* (Blumer, 1926; Bouwens, 1927; Reed, 1908;
Salmon, 1904); *E. valerianae* (Blumer, 1933: 265); *E. artemisiae*
(Neger, 1902); *E. depressa* (Neger, 1902; Blumer, 1922b; Bouwens,
1927); *E. montagnei* (Blumer, 1922b); and *E. horridula* (Blumer,
1922a; Neger, 1902; Bouwens, 1927). If a wider range of hosts had
been employed there is the possibility that such species might have been
shown to have a much wider range of hosts than that cited by Blumer
(1933).

Erysiphe polyphaga is outstanding for its wide host range. Hammar-
lund, and later Blumer, showed that *E. polyphaga* on *Cucumis sativus*
of the Cucurbitaceae infected 85 species of hosts from 15 families. The
cucurbit mildew used by Reed infected primarily certain members of
the Cucurbitaceae and *Helianthus annuus* of the Compositae and *Plan-
tago rugelii* of the Plantaginaceae. Reed inoculated only 3 species of
Aster and *Solidago caesia*, in addition to *H. annuus* and *P. rugelii*, from
families other than the Cucurbitaceae. His mildew might have had a
wider range of hosts if more species had been inoculated. On the other
hand, the cucurbit mildew of the present study was inoculated onto 23
species in 6 families, in addition to members of the Cucurbitaceae.
With this extensive series of host families any tendency for the mildew
to infect species in families other than the Cucurbitaceae would probably
be manifested. It therefore appears that several forms with different
degrees of specialization occur on cucurbits, *E. polyphaga* with its
extremely wide host range, Reed's mildew with two host species other
than in the Cucurbitaceae, and the writer's mildew, restricted to the
Cucurbitaceae. If the present cucurbit mildew is included in *E. poly-
phaga*, one of the primary differences, wide host range, used in separat-
ing the species would not apply to the species as a whole. From its host
specialization it cannot be placed elsewhere with any certainty.

The host range of the zinnia mildew is greater than that for any of
the mildew forms or biologic species under discussion except *E. poly-
phaga*. If the zinnia mildew is included in *E. polyphaga* it would intro-
duce a form intermediate in host range between the mildew recognized
by Hammarlund and Blumer as *E. polyphaga* and several other species.
If included in the other species with which it has hosts in common it
will reduce the differences in host specialization now recognized for them.

The very limited specialization of the phlox mildew to three species
of *Phlox* sharply separates it from other mildews and might be consid-
ered as justification for its recognition as a distinct species. Based on

a preliminary study of perithecial and conidial morphology, it seems evident, however, that the powdery mildews include many forms with various degrees of specialization and with intergrading morphology. In view of the presently available information on host specialization, the slight morphological differences used by Blumer to separate the 10 species from *Erysiphe cichoracearum* are only doubtfully sufficient for recognition of these as distinct species. As far as the mildews used in the present investigation are concerned it seems best to consider them as belonging to one species, *Erysiphe cichoracearum sensu* Salmon, each form being distinguished by its host specialization.

SUMMARY

1. A powdery mildew from *Zinnia elegans* is shown to infect *Z. elegans, Z. pauciflora, Z. verticillata, Helianthus annuus, Arctium minus, A. nemorosum, Xanthium chinense, X. spinosum, X. strumarium, Mikania scandens, Hieracium alpinum, H. prenanthoides, Inula helenium, Carlina acaulis, Lactuca perennis, Cosmos* sp., *Scorzonera hispanica* and *Felicia amelloides* of the Compositae, *Salpiglossis sinuata* of the Solanaceae, and *Cerinthe major* of the Boraginaceae.

2. A phlox mildew is shown to be restricted to *Phlox drummondii* and the cultivated perennial phlox, no infection occurring on 34 other species reported as hosts for *Erysiphe cichoracearum*.

3. A cucurbit mildew from the vicinity of Ann Arbor, Michigan, is shown to be restricted to the Cucurbitaceae, infection failing to develop on all other species inoculated.

4. The zinnia, phlox and cucurbit mildews are best recognized as forms of *Erysiphe cichoracearum* DC. ex Mérat, as recognized by Salmon, distinguished by marked differences in their host specialization.

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OBSERVATIONS ON ASEXUAL AND SEXUAL REPRODUCTIVE STRUCTURES OF THE CHOANEPHORACEAE

ADRIAN W. POITRAS [1, 2]

(WITH 17 FIGURES)

Included in the family Choanephoraceae of the order Mucorales are those genera whose species form their asexual spores both in columellate sporangia and, in addition, either in few-spored, non-columellate sporangioles or in monosporous sporangioles which are commonly referred to as conidia (Hesseltine, **1952, 1953**). Zygospores are produced between the tips of entwining branches.

The aerial sporangium is recognized as the characteristic asexual reproductive structure of the majority of the Mucorales. In certain families and in many genera of this order the sporangium either is accompanied by or is replaced by sporangioles which are small, few-spored, non-columellate sporangia that are often dehiscent at maturity. Monosporous sporangioles often have been regarded as conidia. Investigations reported by Bainier and Sartory (**1913**), Mangin (**1899**), Thaxter (**1914**), Van Tieghem and Le Monnier (**1873**), and Torrey (**1921**) indicate that it is extremely doubtful that a true conidium (as found in the Ascomycetes, Basidiomycetes and Fungi Imperfecti) is produced by any of the Mucorales.

The recognition of the "conidia" found in the Choanephoraceae as monosporous sporangioles, suggested by Thaxter (**1914**), has been generally accepted, but more convincing evidence that an inner spore wall is distinct from an outer sporangial membrane is needed. Other morphological features, such as the occurrence of striations on spore walls and the pattern of sexual reproduction, need additional study, since

these appear possibly to be family characteristics of diagnostic significance.

This investigation was conducted: (1) to establish more clearly the proper interpretation of the "conidia" as found in the Choanephoraceae; (2) to determine whether or not striations are fundamentally important characteristics of all spore types of members of this family; and (3) to ascertain what influence the observations made might have on the taxonomy of the Choanephoraceae.

HISTORICAL REVIEW

Currey (**1873**) placed *Choanephora infundibulifera* in the order Mucedines of the Fungi Imperfecti, implying that he regarded the asexual spores which he observed as true conidia. Cunningham (**1878**) discovered the sporangia, chlamydospores and zygospores of this species and transferred it to the Mucorini (Mucorales). He proclaimed that true conidia are produced by *Choanephora*, thus agreeing with Brefeld (**1872**), who divided the Mucorini into two sub-families, one of which was distinguished by the sporangial fructification, the other by the conidial fructification. In this he opposed the view of Van Tieghem and Le Monnier (**1873**), who denied that the true conidial fructification ever occurs in the Mucorini. Thaxter (**1914**) separated *Choanephora* and *Blakeslea* on the basis of the production of conidia by the former and sporangioles by the latter, but attempted to show that the "conidia" were sporangioles containing a single spore. He failed to demonstrate by mechanical separation an outer sporangial membrane clearly distinct from an inner spore wall, but he was able to show that a thin outer sporangial membrane must exist, for he reported instances when "the contents of the 'conidium' have contracted away from the base of the thin colorless mother cell wall and has surrounded itself with the characteristic purple 'conidial' wall which is clearly distinguished from the empty space below it." Sinha (**1940**) demonstrated the production of single-spored sporangioles by *Blakeslea trispora* when this fungus was cultivated on a "starvation" culture medium, and concluded that these monosporous sporangioles were identical with the "conidia" of *Choanephora*.

Early reports dealing with species of the Choanephoraceae by Currey (**1873**) and Cunningham (**1878**) are devoid of any reference, either by description or illustration, to the presence of striations on the walls of any of the spore types produced by *Choanephora infundibulifera*. Cunningham (**1895**) later described striations on the exospore

walls of the sporangiospores, "conidia" and zygospores of *C. simsonii.*
Thaxter (**1914**) referred only to the "conidia" of *C. cucurbitarum* as
possessing these striations. Lefebvre and Weimer (**1939**) demon-
strated delicate longitudinal striations on the outer sporangiospore walls
of this species and Shanor [3] detected their existence on the zygospores.
In his description of *C. conjuncta,* Couch (**1925**) stated that the walls
of the "conidia" are smooth externally but that the inner spore wall is
apparently minutely punctate. The sporangiospores produced both in
the large columellate sporangia and in the smaller non-columellate spo-
rangioles of *Blakeslea trispora,* according to Thaxter (**1914**), possess
striations which are similar to those observed on various types of spores
of *Choanephora.* The zygospores of this genus, according to Weber
and Wolf (**1927**), are likewise striate and similar to those reported
for *Choanephora.*

The sexual reproductive structures of *Choanephora infundibulifera*
were first illustrated and described by Cunningham (**1878**). He is also
credited with their discovery in *C. simsonii* (Cunningham, **1895**). Wolf
(**1917**) was the first to detect and study zygospores of *C. cucurbitarum.*
Couch (**1925**) described and illustrated the zygospores of *C. conjuncta*
and credits Blakeslee with the discovery of heterothallism in the genus.
Zygospores and heterothallism were described for *Blakeslea trispora* by
Weber and Wolf (**1927**).

<center>MATERIALS AND METHODS</center>

Twenty isolates of *Choanephora cucurbitarum* (Berk. and Rav.)
Thaxter were assembled for this study. Fourteen of these were iso-
lated from 152 soil samples which had been collected in various parts
of Illinois, Mexico, Central America and the island of Ponape. Two
were isolated from the fading flowers of *Hibiscus syriacus* which had
been placed in moist chambers in the laboratory, while the remainder
were supplied by the following: one, isolated from *Nicotiana* sp. in
Sumatra, by the Centraalbureau voor Schimmelcultures, Baarn, Hol-
land; one, isolated from *Cucurbita* sp., by Professor H. L. Barnett,
West Virginia University, Morgantown, West Virginia; one, isolated
from *Cucurbita pepo* var. *melopepo,* by Dr. R. A. Conover, Sub-
tropical Experiment Station, Homestead, Florida; and one, obtained
from the Northern Regional Research Laboratory, Peoria, Illinois,
through the courtesy of Dr. K. B. Raper.

Five isolates representing *Blakeslea trispora* Thaxter were acquired.

[3] Unpublished notes, 1943–1944.

Three of these were obtained from the Northern Regional Research
Laboratory at Peoria, Illinois, and two were received from the Centraal-
bureau voor Schimmelcultures, Baarn, Holland.

Several common synthetic culture media were used in these investi-
gations. The use of these culture media and of specific techniques is
described in appropriate paragraphs on subsequent pages.

OBSERVATIONS AND DISCUSSION

"CONIDIA" OF CHOANEPHORA: "Conidia" of *Choanephora cucurbi-
tarum* were subjected to the following treatments in an attempt to
demonstrate that the outer sporangial membrane may be separated from
the inner spore wall: (1) they were mounted in distilled water; (2)
they were mounted directly in lactophenol with cotton blue; (3) they
were placed in distilled water for varying short periods of time after
which they were mounted in lactophenol with cotton blue; (4) they were
placed in potassium hydroxide and permitted to swell after which they
were mounted in lactophenol with cotton blue; and (5) they were placed
in saline solution for varying short periods after which they were
examined.

The "conidia" subjected to the first four of these treatments were
only slightly modified. Most of the treated "conidia" that were ob-
served resembled the true exogenous conidium of the higher fungi; their
endospores completely filled the sporangiolic membranes. "Conidia"
which contained endospores that did not completely fill their sporangiolic
membranes were also observed (FIGS. 1–5). In certain of these in-
stances, "conidia" resembling those described and illustrated by Thaxter
(**1914**) were detected (FIGS. 1, 4). While some contraction of the
endospores in these "conidia" may have occurred, it was evident that
the spores had failed to mature and conform to the size and shape of
the outer sporangiolic membranes. "Conidia" which exhibited "empty"
spaces within the sporangiolic membrane at both the basal and apical
ends of the endospore were also not infrequently encountered (FIGS.
2, 3, 5).

Those "conidia" which were subjected to the saline solution became
extremely plasmolized and unsuitable for study.

In an additional attempt to demonstrate an outer thin wall of the
sporangiole and a separate colored and striate inner wall of the endo-
spore, "conidia" were placed in drops of sterile water on the surface
of nutrient agar in Petri dishes so that germination could be observed.
When a majority of these "conidia" under observation produced germ
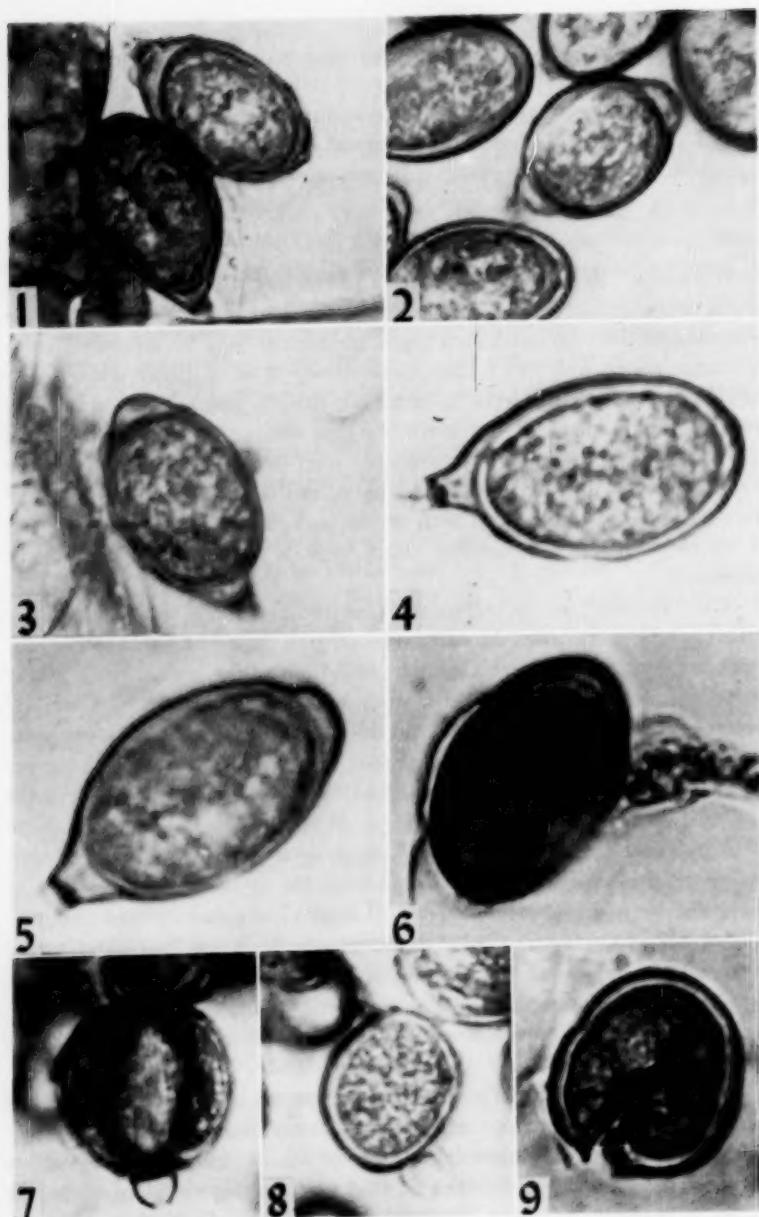
FIGS. 1–9.

tubes, they were transferred to lactophenol on microscope slides for examination. In a few instances germinating "conidia" were detected in which the outer thin sporangiolic membrane was drawn out for a short distance with the germ tube. This indicated clearly that the inner spore wall is separate and distinct from the outer sporangiolic membrane (Fig. 6).

In all cases the outer sporangial membrane was found to be of a thin, membranous texture and to be uncolored, while the endospore wall was thick and purplish-brown. The thick endospore wall was also found to possess longitudinal striations. In no instance was it possible to demonstrate that these endogenous spores of the "conidia" possess terminal clusters of fine radiating appendages as are found on the sporangiospores of the multi-spored sporangia of *Choanephora* and *Blakeslea*.

SPORANGIOLES OF BLAKESLEA: In an effort to determine the significance of the number of sporangiospores per sporangiole in *Blakeslea trispora*, this fungus was cultivated on an agar medium of low nutritional level, the same as used by Barnett and Lilly (**1950**) in a study of asexual reproduction of *Choanephora cucurbitarum*. Two interesting phenomena were observed: (1) the number of sporangiospores produced in the sporangia was reduced, many being observed with only two or three spores and single-spored sporangia were not uncommon (Fig. 11); (2) the number of sporangioliferous heads on any one sporangiophore was fewer and the number of sporangiospores produced in the sporangioles which developed on these sporangioliferous heads was likewise smaller. Many sporangioles with three or more spores developed in a normal manner (Fig. 7). In many instances, true monosporous sporangioles were observed (Fig. 8). In addition, abnormal sporangioles with single spores were also found (Fig. 9). In this study no attempt was made to explain this anomolous condition and it seems best to regard these as curious abnormalities. All of the monosporous sporangioles observed were distinctly double-walled structures, having a thin, colorless outer sporangial membrane and a thick, purplish-brown inner spore wall.

FIGS. 1–6. Single-spored sporangioles of *Choanephora cucurbitarum*. 1–5. Sporangioles whose endospores do not completely fill the spaces within their sporangiolic membranes, × 1800–2000. 6. Germinating sporangiole showing separation of inner spore wall and outer sporangiolic membrane, × 2000. FIGS. 7–9. Sporangioles of *Choanephora trispora*. 7. Typical multiple-spored sporangiole, × 1750. 8. Single-spored sporangiole, × 1750. 9. Abnormal single-spored sporangiole, × 1875.
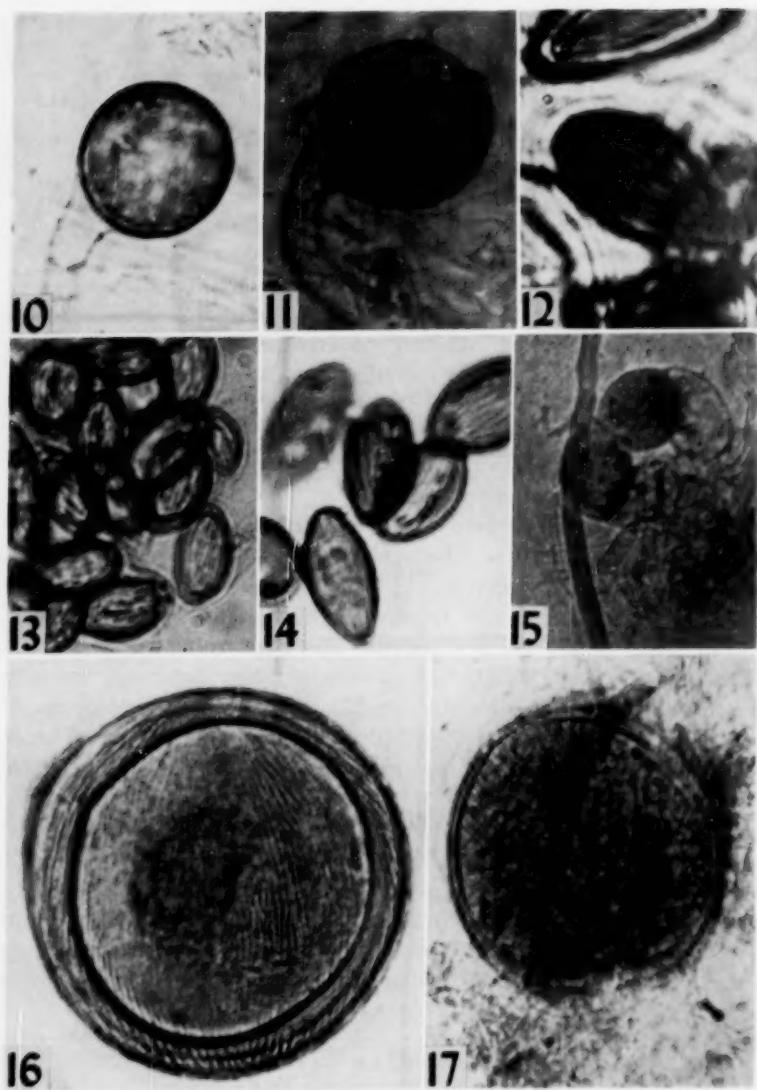
Fig. 10. Single-spored sporangium of *Choanephora cucurbitarum*, × 1150.
11. Single-spored sporangium of *C. trispora*, × 1200. 12. Striate sporangiospore
of *C. cucurbitarum*, × 1600. 13. Striate sporangiospores of *C. trispora*, × 500.
14. Striate endospores of single-spored sporangioles ("conidia") of *C. cucurbitarum*,
× 1200. 15. Typical gametangia of *C. cucurbitarum*, × 200. 16. Striate zygospore
of *C. cucurbitarum*, × 700. 17. Striate zygospore of *C. trispora*, × 800.

SPORANGIA OF CHOANEPHORA AND BLAKESLEA: In culture, isolates
of both *Choanephora* and *Blakeslea* develop large, columellate, pendent
sporangia containing many sporangiospores. Barnett and Lilly (**1950**)
have shown that this type of sporangium is produced more abundantly
by *Choanephora* when it is cultivated on a "starvation" substrate, espe-
cially when the incubation temperature is maintained at about 30° C.
By duplicating the conditions described by Barnett and Lilly for their
experiments with *Choanephora*, it was possible to confirm their results.
When *Blakeslea* was subjected to the same conditions, results were simi-
lar to those obtained with *Choanephora*. In both, in addition, smaller
few-spored sporangia which lacked columellae were abundant. These
were found intermingled with the larger columellate sporangia in the
same cultures. In certain cases, minute sporangia lacking columellae
and containing one, two or more sporangiospores were observed (FIGS.
10, 11). These appeared to possess the characteristics of sporangio-
spores developed in the larger columellate sporangia. No significant
differences were observed in the sporangia produced by these two
organisms.

SPORE STRIATIONS: Several spore types of the different species of
Choanephora and of *Blakeslea trispora* have been reported to be striate.
In view of the apparent universality of spore striation and its possible
significance as a diagnostic characteristic of the Choanephoraceae, the
occurrence of striations on the walls of all available spore types produced
by these fungi was investigated.

The walls of sporangiospores of *Choanephora cucurbitarum* were
studied through the compound microscope with combinations of lens
which gave magnifications of 1080–1800 diameters. Extremely fine and
delicate longitudinal striations which frequently anastomosed were read-
ily detected on the walls of these spores (FIG. 12). Examination of
preserved specimens of *C. conjuncta* borrowed from Professor John
Couch revealed that the walls of the sporangiospores of this species
likewise are striate. When the walls of sporangiospores produced by
Blakeslea trispora were studied in a similar manner, the same charac-
teristic striations were readily distinguishable (FIG. 13).

Couch (**1925**) refers to the "conidia" of *Choanephora conjuncta* as
possessing smooth outer walls. He stated that the inner walls are
minutely punctate. Examination of Couch's specimens of *C. conjuncta*
lead us to conclude that the "conidia" of this species are not sig-
nificantly different from those produced by *C. cucurbitarum*. Striations,
although faint and difficult to detect, are present on the "conidial" walls
and are similar to those present on the "conidia" of *C. cucurbitarum*.

Irregularly spaced refractory granules of unknown nature were observed around the periphery of the endospores, and these may represent the punctations reported by Couch. Striations on the endospore walls of the "conidia" of *C. cucurbitarum* are illustrated in Fig. 14.

The possession of finely striate exospore walls by the zygospores of *Choanephora simsonii, C. conjuncta* and *Blakeslea trispora* has been reported by Cunningham (**1895**), Couch (**1925**) and Weber and Wolf (**1927**), respectively. Shanor [a] observed that the exospore walls of zygospores of *C. cucurbitarum* are also finely striate. Restudy of Shanor's specimens and of other isolates of this species has confirmed this observation. These striations (Fig. 16) are similar to those detected on the walls of the "conidia" and the sporangiospores of *C. cucurbitarum*. Examination and study of zygospores of *C. conjuncta* revealed that the zygospores of this species are similarly striate. The striations on the zygospores of *C. cucurbitarum* originate in the region adjacent to the suspensors and spread over the spore as longitudinal, frequently anastomosing lines.

Zygospores of *Blakeslea trispora* which were borrowed from Dr. C. W. Hesseltine were carefully examined. Characteristic striations were identified on the walls of these (Fig. 17), thus confirming the report of Weber and Wolf (**1927**). These striations are similar to those observed on the walls of zygospores of *Choanephora cucurbitarum* and *C. conjuncta*.

Morphology of Sexual Reproduction : The method of zygospore production by species of the Choanephoraceae was investigated and found to be in accord with the descriptions by Couch (**1925**), Shanor [a] and Wolf (**1917**). In all instances among currently recognized members of this family (Hesseltine, **1952, 1953**), zygospores are produced following the fusion of gametangia which are cut off from the tips of twining branches of fertile hyphae (Fig. 15). The extent of the complex entanglements of vegetative hyphae associated with the production of gametangia and zygospores was found during the course of this study to be a condition which varied, apparently, with the nutritional level of the substrate and mating vigor. This indicates that the extent of the entanglement of vegetative hyphae is a matter of little significance and unlikely of any diagnostic importance.

TAXONOMIC CONCLUSIONS

This investigation of the morphology of "conidia" of *Choanephora* has demonstrated that these spores possess double walls, an outer, thin

sporangial membrane and an inner, thick, true spore wall. Thus these "conidia" are clearly monosporous sporangioles that differ from the sporangioles of *Blakeslea trispora* only in the number of spores produced.

At the time he established the genus *Blakeslea*, Thaxter (**1914**) stated that the only fundamental difference between *Blakeslea* and *Choanephora* was the production of sporangioles by the former and the production of "conidia" by the latter. Results of this investigation of the "conidia" of *Choanephora* and the single-spored sporangioles of *Blakeslea*, and of the similarity of their sporangia, support the conclusion that their differences are not of generic significance, but rather, only of specific importance. Sinha's conclusion that the generic name *Blakeslea* must be considered a synonym of *Choanephora* is, therefore, regarded as valid. The correct epithet for the species having multi-spored (usually 3–5) sporangioles is *Choanephora trispora* (Thaxter) Sinha.

Careful examination of all spore types produced by *Choanephora cucurbitarum, C. conjuncta* and *C. trispora* has resulted in the detection of fine, delicate striations on their walls in very instance. These observations confirm reports of striations on the walls of spores produced by members of this family. Whether the walls of the different spore types of *C. infundibulifera* are striate or not is unknown since neither Currey (**1873**) nor Cunningham (**1878**) has referred to this characteristic, and since no material representing this species was available for study. It seems likely, however, that the presence of striations on the walls of spores of the Choanephoraceae should be looked upon as a family characteristic of diagnostic importance.

The formation of zygospores between the tips of twining branches of fertile hyphae has been shown to be the characteristic method of zygospore development in the Choanephoraceae. This method of zygospore development is not restricted to the Choanephoraceae, but when it is considered along with such additional morphological characteristics as striations on the walls of all spore types and the production of aerial sporangia and sporangioles on separate sporangiophores, it serves to distinguish them from other fungi assigned to the Mucorales.

This investigation has revealed that the "conidia" of *Choanephora conjuncta* are not unlike those produced by *C. cucurbitarum*. The formation of "elaborate compilations of hyphae" at the base of the zygospores of *C. cucurbitarum* is directly related to the nutritional level of the substratum and the mating vigor of the opposite mating type strains. Therefore, this phenomenon, as reported for *C. conjuncta*, should not be regarded as a specific diagnostically important characteristic. In other

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features, *C. conjuncta* agrees with *C. cucurbitarum*. It is concluded, therefore, that there is insufficient evidence to support the retention of *C. conjuncta* as a valid species and that this name should be included as a synonym of *C. cucurbitarum*.

SUMMARY

Twenty isolates of *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter and five designated *Blakeslea trispora* Thaxter were obtained from soil and other sources. Experimental studies were conducted and observations made on the fundamental morphology of "conidia" and sporangioles. The "conidia" of *Choanephora* are truly monosporous sporangioles and similar, except for the number of spores developed in each, to sporangioles of isolates designated as *Blakeslea*. Striations have been observed on the walls of all spore types produced by all of the isolates of the Choanephoraceae investigated. This characteristic appears to be of fundamental diagnostic importance for the family.

The formation of zygospores between the tips of twining branches of fertile hyphae is the characteristic method of zygospore development in the Choanephoraceae. This type of zygospore formation and other morphological characteristics, such as spore striations and the production of aerial sporangia and sporangioles on separate sporangiospores, combined, distinguish species belonging to the Choanephoraceae and separate them from other fungi belonging to the Mucorales. Observations reported here substantiate Sinha's proposal that the principal difference between *Blakeslea* and *Choanephora* is not of generic significance and, therefore, *Blakeslea* must be considered a synonym of *Choanephora*. *Choanephora conjuncta* is concluded to be a synonym of *C. cucurbitarum*.

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CAPILLITIAL DEVELOPMENT IN THE MYX-
OMYCETES BADHAMIA GRACILIS AND
DIDYMIUM IRIDIS

Arthur L. Welden

(with 3 figures)

Probably the earliest investigations into the nature of the capillitial threads of Myxomycetes were conducted by Strasburger (**10**). In his work on *Trichia fallax* [*T. pusilla* (Hedw.) Martin],[1] he described the capillitium as forming in vacuoles found in the cytoplasm of the developing sporangium. The sequence of development as he described it was: vacuoles are formed, elongate, anastomose, become tubular in shape and, by deposition of granules along their length, acquire their characteristic sculpturings. Strasburger demonstrated for the first time that capillitial threads are not elongated cells, but are formed as the result of intraprotoplasmic secretions.

Zopf (**12**), while accepting Strasburger's work with hollow threads, believed the so-called solid capillitial threads to represent plasma masses not used in spore formation. Zopf did not present any verification of this viewpoint. Massee (**9**) either ignored or overlooked Strasburger's work when he maintained that the capillitium arose from surplus protoplasm left after spore formation which takes the form of a more or less complicated network mixed with the spores. Kräntzlin (**6**) derived the capillitium of *Oligonema* and *Arcyria* from "elateroplasts." These structures were described as arising from unipolar spindles formed as a result of division of certain nuclei. The rays or spindle fibers of the heteropolar spindle tended to disappear as the full development of the vacuoles was approached.

Harper and Dodge (**4**) confirmed Strasburger's work and accepted his interpretation that the capillitium is an intraprotoplasm secretion. Working with *Hemiarcyria clavata* [*Hemitrichia clavata* (Pers.) Rost.] and a species of *Trichia*, they found that capillitial formation is inaugurated as soon as the sporangium attains full size. Vacuoles are formed in the protoplasm of the developing sporangium, elongate and anastomose and, at first, resemble a physaroid capillitium. Ultimately, these vacuoles

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[1] Names in brackets are according to modern nomenclature (see Martin, **8**).

assume the characters of the mature trichioid capillitium. The proto-
plasm is slightly more condensed around these vacuoles and is, in turn,
surrounded by a zone of nuclei. The nuclei away from the vacuoles
show no such orientation. Fibrils are seen to run amongst the nuclei
and connect with the numerous granules which lie upon the surface
of the vacuoles. These fibrils are not connected to the nuclei and tend
to disappear as the capillitial threads mature. Harper and Dodge
believed these fibrils to be what Kräntzlin called heteropolar spindles.

A different interpretation of capillitial development is given by
Bisby (1). According to him, the capillitium arises as invaginations
from the surface of the peridium. In *Physarella mirabilis* [*P. oblonga*
(B. & C.) Morg.] these invaginations form from the peridial surface
and cut into the protoplasm, forming the capillary tubes and line knots
found in the mature fructification. Invaginations occur also from the
columella surface in *Stemonitis fusca* and join those invaginations from
the peridial surface. Cadman (2) maintains that, in the case of *Didym-
ium nigripes* Fr., the capillitium forms as the result of condensation
along the interface between the cytoplasm of the sporangium and the
invaginations into it from the peridial surface. This condensation, she
claims, is due to water loss and results in the formation of capillitial
threads.

A radically different explanation for capillitium formation is given
by Wilson and Cadman (11). They report for *Reticularia Lycoperdon*
Bull. that certain tracts of protoplasm degenerate, giving rise to the
capillitium. *Reticularia* has often been said to have a pseudocapillitium
and this type of development may well be the basis of the morphological
distinction between a true capillitium and a pseudocapillitium. Howard
(5) agrees with Harper and Dodge that the capillitium is an intra-
protoplasmic secretion, but, unlike those workers, he reports that the
physaroid capillitium arises after the tubular elongations occur.

MATERIALS AND METHODS

Plasmodia of *Badhamia gracilis* (Macbr.) Macbr. and *Didymium
Iridis* (Ditmar) Fries were obtained by moistening dead *Yucca* leaves
and bark, using the method described by Gilbert and Martin (3). The
Yucca leaves were collected by Mrs. Calvin McMillan in Caliente,
Nevada, on December 28, 1950, and were wet October 12, 1953. Fruit-
ing bodies of the *Badhamia* began to appear about one week after wetting.
They were removed from the plant parts over an extended period of time.
The bark upon which *Didymium Iridis* appeared was collected on Barro

Colorado Island, Canal Zone, Panamá, on July 5, 1952. It was wet
on August 22, 1953, and subcultured on oat agar many times.

At the initiation of fruiting, the protoplasm of the plasmodium flows
into the spreading fans and gathers into pulvinate masses. These masses
subsequently elongate and form mature fructifications. As the pulvinate
masses began elongation, they were killed in a variety of fixatives. All
subsequent development stages were also fixed until as nearly a com-
plete series as possible was obtained. Of the fixatives used, the most
satisfactory were Fleming's Weaker Solution and Navashin's fixative.
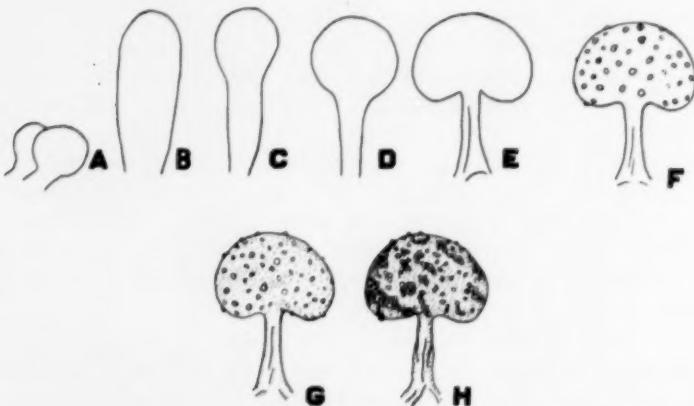
FIG. 1. A–H. Schematic diagrams of the developing sporangia of *Badhamia
gracilis* and *Didymium Iridis* from pulvinate initials to the mature sporangium.
A. Pulvinate initials. B. Columnar initials formed after the elongation of the
pulvinate initials. C–E. Apical expansion of the initial with a corresponding
reduction in the diameter of the stipe. F. First appearance of calcium carbonate
upon the peridium, which signifies that the capillitium is mature and functioning.
G. Stage of first color changes in the maturing sporangia. H. Mature sporangium.

This was especially true if the modified triple stain described by Johansen
(*Plant Microtechnique*, p. 86) was used. After fixing for 24–48 hours,
the material was dehydrated by the Butyl alcohol method, placed in
paraffin oil, and embedded in paraffin. Sections were cut 8μ thick,
mounted on slides and stained.

RESULTS

The heaping of the plasmodial protoplasm into numerous small,
pulvinate masses forming what is here termed the "sporangial initials"

is the first indication of fruiting.   Shortly after these initials are formed,
they undergo an elongation and form the "columnar initials."   The
apex of the column is slightly larger than the base.   The apical end
expands laterally, forming the globose to subreniform sporangia char-
acteristic of the two species.   As lateral expansion increases, the proto-
plasm of the stipes empties into the sporangia and the stipes are gradually
reduced in diameter.   Fig. 1, A–H, shows a schematic representation
of this growth pattern.   During this period of gross morphological
change, there does not appear to be any increase or decrease in the
quantity of protoplasm, but only a change in the volume occupied by
the protoplasm due to condensation.

Certain color changes occur in the protoplasm of the two species
during maturation.   To some extent these color changes can be cor-
related with cytological development.   At first the plasmodia of both
species are milk-white and this color persists until maturation is almost
complete.   Toward the latter part of the stage corresponding to Fig.
1, F, calcium carbonate granules appear on the surface of the sporangia,
which indicates the cytological stage at which the capillitium has com-
pletely formed and is functioning.   The appearance of calcium car-
bonate also denotes that spore cleavage is beginning, for this occurs
immediately after capillitial formation.   At first the calcium granules
are not noticeable, but, as the sporangia change color, they become more
prominent.   In *B. gracilis* the first color change is to pale-pink which
becomes progressively darker through lavender to purple.   In *D. Iridis*
the change is to pale brownish-pink which finally becomes dark purple.
As the colors of the sporangia change and darken, spore cleavage occurs
and the sporangia attain maturity.

In both species, the first stage of the developing sporangia which
was fixed was immediately after the pulvinate initials had elongated
and formed the columnar structures.   Thereafter, the sporangia were
fixed at hourly and half-hourly intervals until as nearly a complete series
as possible was obtained.   Both *B. gracilis* and *D. Iridis* are similar in
many respects, but certain differences are evident and, for this reason,
the two species will be discussed under separate headings.   *B. gracilis*
will be discussed in greater detail and *D. Iridis* compared with this
development.

BADHAMIA GRACILIS

In Fig. 2, the developmental sequence is shown by means of photo-
micrographs, and also semi-schematically to demonstrate more clearly
the points discussed.
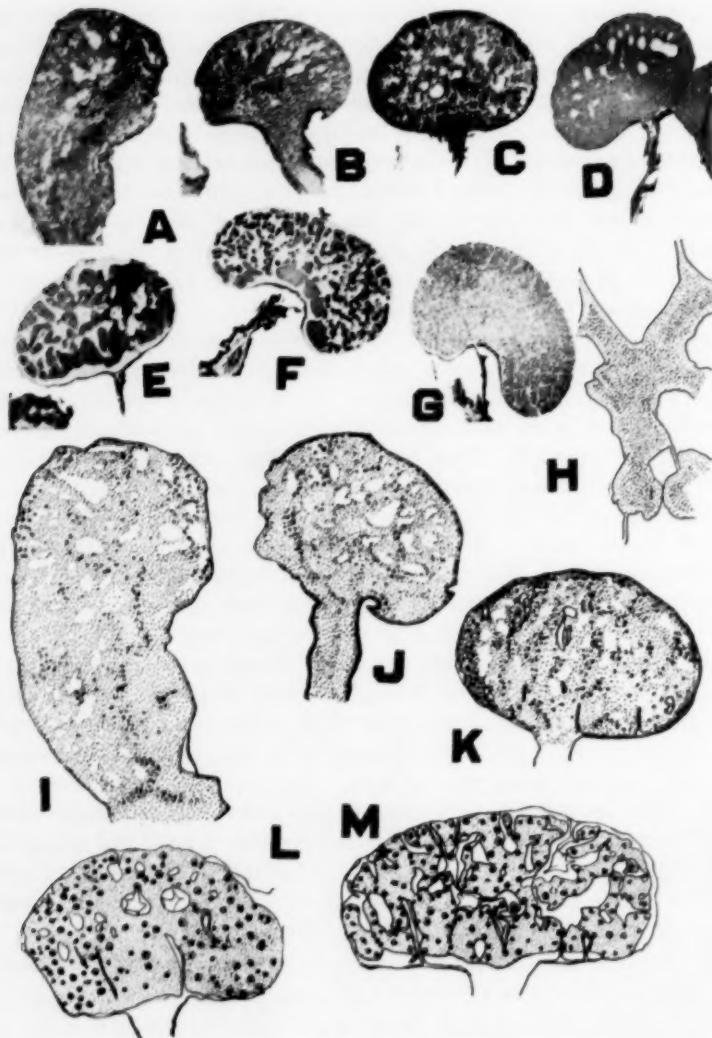
FIG. 2. A–H.

FIG. 2. A–G. Photomicrographs of sections of the developing sporangium of *Badhamia gracilis*. A. Columnar initial showing the vacuolate condition of the protoplasm. B. Expanded apex of the initial. C. Sporangium, showing the beginning of peripheral condensation of the protoplasm. D. Sporangium containing the large capillitial vacuoles and the invaginations into the protoplasm from the peridium. E. Mature capillitium and the manner in which it cuts the protoplasm

FIG. 2, A and I, show that the columnar initial is composed of reticulated protoplasm. Toward the base of the column, which is smaller than the apex, the protoplasm contains a few relatively small vacuoles, with larger more numerous vacuoles toward the apex. Many variously shaped and darkly staining nuclei are dispersed throughout the protoplasm. At first glance, these nuclei seem to be randomly distributed but, upon closer examination, certain irregular lines of nuclei can be seen. These lines of nuclei probably represent streams of protoplasm flowing into the elongating initials. The more or less spherical nuclei measure $3-4\,\mu$ in diameter but some are irregular in shape. Crystal Violet stains all of the nuclei deeply. When stained for an equal time at this and at later stages, it was found that the nuclei, for some unknown reason, retain Crystal Violet more tenaciously at this stage. If subjected to destaining procedures, the nuclei decolorize slowly, but at no time is there any structure discernible within them. When the nuclei are stained with Haemotoxylin and destained in Iron Alum similar results are obtained. There does not seem to be any evidence of nuclear disintegration or disorganization.

In addition to nuclei, many darkly staining granules are present which tend to disappear at later stages. These granules are probably excretory products which are being expelled by the heaping protoplasm. The only indication of a surrounding wall is a slight condensation of the protoplasm around the apex. This membrane thickens considerably toward the base. Exposure to air is probably the cause of this condensation.

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into large multinucleate masses. F. Sporangium after a certain amount of cleavage has occurred, demonstrating that cleavage is more or less progressive from the periphery to the interior. G. Mature sporangium. H. Camera lucida drawing of a portion of the mature capillitium. I–M. Schematic representation of fruiting in *Badhamia*. The outlines of the initials and sporangia were drawn with the aid of a camera lucida. The nuclei are enlarged considerably to show their orientation. I. Columnar initial showing the vacuolate condition of the protoplasm and the streams of nuclei and traces of peripheral condensation. K. Section of a sporangium demonstrating the definite beginnings of peripheral condensation, the vacuolate protoplasm (some of the vacuoles with visible membranes) and the streams of nuclei. Tubular invaginations are forming from the periphery. L. Sporangium after condensation of the protoplasm is complete. Capillitial vacuoles are present, tubular invaginations have occurred and anastomosed with the vacuoles. All the nuclei show a nucleolus, chromatin strands and a nuclear membrane. Some nuclei are in individual vacuoles. M. Multinucleate masses of protoplasm at the beginning of spore cleavage. Most of the nuclei are in the metaphase. The capillitium is mature and functioning. A–G. Photomicrographs, approximately × 39; H–M. Drawings, approximately × 33.

At a later stage (FIG. 2, B and J), the sporangial mass and stipe have been delimited. The interior of the stipe is still filled with protoplasm, but it is slowly becoming enucleate. The protoplasm in the stipe will ultimately become transported into the sporangium or will disintegrate. The outermost portion of the sporangium is surrounded by a slightly thickened membrane. Interior to this membrane is an area of condensed protoplasm which, with the external membrane, will make up the peridium. Filling the remainder of the sporangium inside the condensed protoplasm is the frothy, uncondensed reticulum.

Still later (FIG. 2, C and K), the stipe has been fully differentiated and is enucleate. Occasional masses of disintegrating protoplasm may sometimes be seen in the interior of the stipe. The peridial zone of the sporangium is more pronounced. By careful focusing around the periphery and base of the sporangium, tubular invaginations can be seen forming from the inner wall of the now double-walled sporangium and pushing into the interior. These invaginations stain only slightly darker with Orange G than the surrounding protoplasm and are, therefore, easily overlooked. Inside the peridial zone a large area of condensing protoplasm can be seen to be increasing in size. Two types of nuclei are present in this zone: (1) the first type is the heavily staining nuclei which were described above, and (2) lightly staining nuclei which appear for the first time. This second type does not retain any Crystal Violet or Safranin, but stains heavily with Orange G. The interior of the sporangium is still filled with frothy protoplasm containing streams of heavily staining nuclei. Frequently, vacuoles with membranes appear toward the periphery of this frothy protoplasm. Other vacuole-like structures without visible membranes are dispersed throughout the protoplasm.

Condensation of the protoplasm continues in the later stages. It is soon obvious that the pattern of condensation is from the periphery to the interior of the sporangium. As peripheral condensation occurs there is a corresponding reduction in the amount of central, frothy protoplasm. This condensation or gelling of the protoplasm continues until the interior of the sporangium is filled with a homogeneous mass. During condensation, water and waste products are excreted into vacuoles formed in the protoplasm. These vacuoles, which are invested with clearly visible membranes, are the capillitial vacuoles. Presumably one of the waste products is calcium carbonate and, as the vacuoles elongate slightly, anastomose with each other and the tubular invaginations, it is transported to the exterior of the sporangium through the invaginations. Some of the calcium products remain in the vacuoles

and tubes, thus forming the more or less physaroid capillitium characteristic of *B. gracilis* (FIG. 2, H).

Sections of the sporangium after the capillitium is formed (FIG. 2, D and L), show the homogeneous mass of protoplasm containing large vacuoles, 15–17 μ in diameter, connected with each other and with the capillary invaginations. The peridium is double-walled and the stipe is completely differentiated. The only further changes these structures will undergo is slight shrinkage as the sporangium dries out after spore cleavage.

The formation of capillitial threads has divided the protoplasmic mass into numerous large, multinucleated masses of what can now be called the sporoplasm (FIG. 2, E and M). The nuclei at this stage present a very different picture than in previous stages. It seems that as protoplasmic condensation continues, the nuclei undergo some change and they no longer retain the Crystal Violet as before, but present the typical nucleus picture. They are found to contain a red-staining nucleolus and blue-staining chromatin strands surrounded by an orange-staining nuclear membrane. With very few exceptions, only this type and those which stain only with Orange G are found in the condensed protoplasm. The reason for this change in staining properties is unknown. Perhaps, since nuclear divisions will begin shortly, this change indicates the beginning of a prophase.

The multinucleated masses of sporoplasm left from capillitial formation begin to be cleaved into smaller and smaller ones. The cleavage furrows are formed on the surface of the sporoplasm bounding the capillitium and cut into the large protoplasmic masses. Throughout cleavage the nuclei are in various division stages and there is some indication, as yet unsubstantiated, that these divisions are meiotic in nature. As cleavage continues it is noticeably more advanced toward the peridial surface than toward the interior (FIG. 2, F and M). When it is complete, the spores lie pressed together and are polyhedral in shape. Two nuclei are often seen in some of the spores. This would indicate that an occasional cleavage furrow often overtakes a dividing nucleus and encloses it in a single spore. As the spores pull away from one another and assume their spherical shape, the characteristic spore markings are formed. This pulling apart is undoubtedly caused by the drying of the now mature sporangium. The nuclei of the spores are 3–4 μ in diameter.

FIG. 2, G is a photomicrograph of a mature sporangium showing the reniform appearance it has in section. The sporangium is filled with warted spores and physaroid capillitial threads.
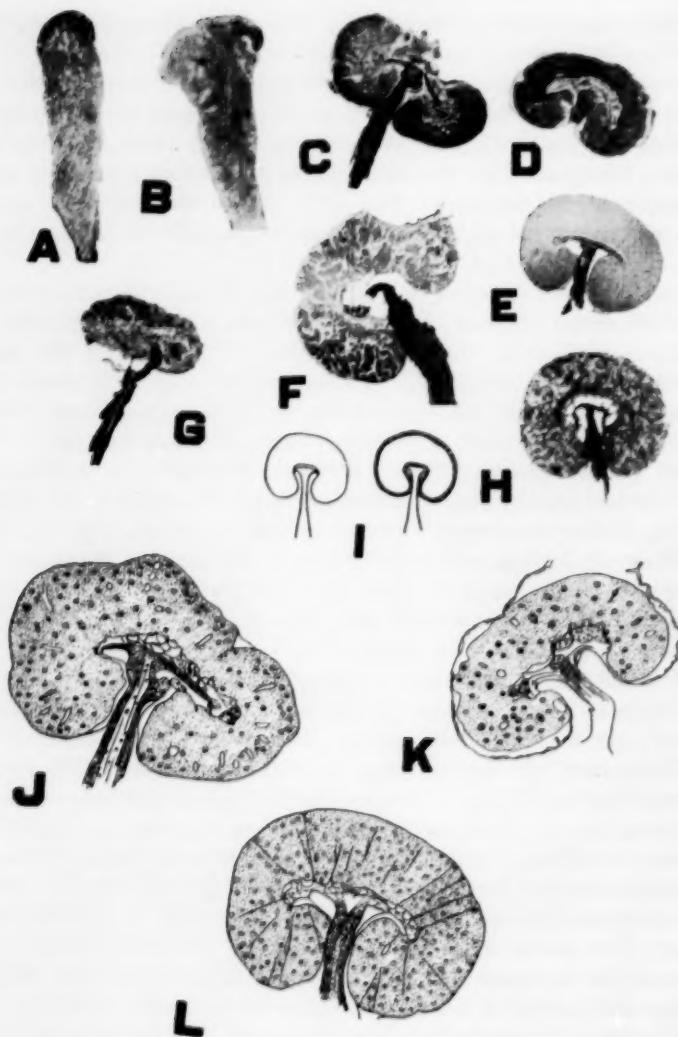
Fig. 3.   A–H.

Fig. 3.   A–H.   Photomicrographs of sections of the developing sporangium of *Didymium Iridis*.   A. Elongate columnar initial filled with vacuolate protoplasm. B. Initial as it begins lateral expansion at the apical end.   C. Sporangium after lateral expansion of the initial has ceased, showing how the columella is formed by the outward and downward expansion of the sporangium.   A few small vacuoles are present and the first traces of peripheral protoplasmic condensation can be

DIDYMIUM IRIDIS

The external morphological changes which *D. Iridis* undergoes during fruiting are identical with the changes in *B. gracilis*. As nearly as possible stages of this species similar to those of *B. gracilis* were fixed and sectioned. Fortunately, plasmodia of *D. Iridis* can be maintained easily on oat agar and thus a more complete series of developing sporangia could be obtained.

The first stages of the development of the initials are similar in every respect to those of *B. gracilis* (FIG. 3, A). However, the protoplasm is not as reticulated, nor do the nuclei retain Crystal Violet as tenaciously. The first major change from the pattern described for *B. gracilis* appears during the lateral expansion of the sporangium. Unlike *B. gracilis*, the sporangium of *D. Iridis* expands downward as well as laterally and encloses a portion of the stipe (FIG. 3, C–E, H–L). As subsequent stages prove, it is in this manner that the columella of *D. Iridis* develops. An area immediately above the enclosed stipe and occupying approximately one-third or less of the sporangium is separated from the remainder of the protoplasm by a thickened membrane (FIG. 3, D and J). Interior to this membrane, the area is filled with pieces of protoplasm which appear to be disintegrating. As the sporangium develops, this protoplasm is replaced by what appear to be frayed membranes and is more or less hollow (FIG. 3, D, J and K). In cross-

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seen immediately interior to the peridial area. A portion of the columella area can be seen. D. Section of a sporangium after complete condensation of the protoplasm, showing the small capillitial vacuoles, the columella space and the invaginations into the protoplasm. E. Section through a sporangium after the capillitium is mature. F. Cleavage of the protoplasm after capillitial formation, showing that cleavage is more advanced adjacent to the columella area and at the periphery than in the interior. G. Sporangium after cleavage has progressed until only one or two more cleavages will delimit the spores. H. Section of a mature sporangium showing the spores and columella. I. Schematic diagram showing how the columella develops. The shaded area is where lime from the condensing protoplasm is deposited. J–L. Schematic diagrams representing stages in the developing sporangium of *Didymium*. The outlines were drawn with the aid of a camera lucida. Nuclei are greatly enlarged and younger stages are omitted because of their similarity to *Badhamia*. J. Sporangium containing the small capillitial vacuoles and the developing columella space which is more or less filled with disintegrating protoplasm. The peripheral condensation of protoplasm is beginning. The nuclei show only a nucleolus and a nuclear membrane. K. Sporangium after complete condensation of the protoplasm, which is being invaded by tubular invaginations from the columella space and from the peridium. L. Sporangium with mature capillitial threads. A–H. Photomicrographs, approximately × 39; J–L. Semi-schematic drawings, approximately × 33.

section this area and the enclosed stipe appear hollow. As the sporangium develops further this space becomes filled with calcium carbonate and contributes to the formation of the columella (see FIG. 3, I for a schematic representation of columella development). Henceforth, this area will be referred to as the columella space.

During the expansion of the sporangium and the formation of the columella space, the protoplasm of the sporangium proper condenses. Tubular invaginations from the peridial wall cut into the protoplasm (FIG. 3, D and J). Protoplasmic condensation in *D. Iridis* is much more rapid than in *B. gracilis*, probably due to the large columella space which provides an additional large surface from which evaporation can take place. No large capillitial vacuoles such as are found in *B. gracilis* are present in *D. Iridis*. Again, this is probably due to the presence of the columella area which replaces the vacuoles in functioning as an area of deposit of water and waste products. A few small vacuoles are formed, however, and these are no doubt responsible for the occasional nodular swellings found on the mature capillitial threads.

The tubular invaginations not only form from the peridial surface but also from the surface of the columella space (FIG. 3, D, E and K). These invaginations anastomose with each other obliquely, giving the appearance of branching in the mature threads. They also connect with the opposite surface, *i.e.*, peridial invaginations with the membrane of the columella space and the columella invaginations with the peridial surface. These numerous tubules rapidly carry away water and waste products excreted by the condensing protoplasm to the sporangial surface and to the columella space. This factor is undoubtedly the major reason why *D. Iridis* has a calcium-free capillitium. At maturity and upon drying, the sides of the empty capillitial threads either wholly or partially collapse, giving the appearance of solid threads.

After capillitial formation the columella space is filled with calcium carbonate, the surface of the sporangium is covered with this product and the protoplasm—now called the sporoplasm—has been cut into large, multinucleate masses by the capillitium (FIG. 3, F and L). Immediately after this stage spore cleavage begins. As in *B. gracilis* cleavage is accompanied by nuclear divisions which appear to be meiotic divisions. In *B. gracilis* the cleavage furrows are first formed at the surface of the peridium and cleave progressively inward. However, in *D. Iridis* furrows first form not only at the peridial surface but also at the surface of the columella space (FIG. 3, F). The area between the columella and the peridium is the last to be cleaved into spores. This would seem to indicate that cleavage furrows form at the surfaces

where evaporation is occurring.   Cleavage in *D. Iridis* is more regular than in *B. gracilis,* for only rarely is a dividing nucleus included in a spore.   After cleavage is complete the spores, like those of *B. gracilis,* lie closely appressed and are polyhedral in shape (FIG. 3, G).   As the spores pull apart the characteristic spore markings appear.

FIG. 3, H is a photomicrograph of a section through a mature sporangium of *D. Iridis* and shows the columella space, giving some indication of how it formed.   The mature spores and an occasional capillitial thread can also be seen.

<center>DISCUSSION OF RESULTS</center>

The results presented here for capillitial formation in the calcareous species *Badhamia gracilis* and *Didymium Iridis* agree in most details with those presented by Bisby (**1**) for *Physarella mirabilis* and *Stemonitis fusca* and those of Cadman (**2**) for *Didymium nigripes.*   Bisby prefers to call the vacuoles in *Physarella* lime-knots and implies that they are formed by capillary invaginations into the protoplasm.   I cannot agree with that interpretation; the lime-knots seem to be formed as a result of the excretion of calicum compounds into the vacuoles resulting from protoplasmic condensation.   The manner in which the vacuoles of *Badhamia* and *Didymium* are formed is substantially the same as in *Hemiarcyria* and *Trichia* as described by Harper and Dodge (**4**).   In the former species, however, waste compounds are excreted into the vacuoles rather than deposited on the vacuolar surface.   The capillitial threads of *Badhamia* and *Didymium* were formed in a different manner from those of *Hemiarcyria* and *Trichia.*   Since the latter species are considered to be in a different taxonomic category, this might prove to be an additional evidence for maintaining their separation in different orders.   Howard (**5**), in his work on *Physarum,* reports that a capillitium of elongated vacuoles of equal diameter characteristic of some species of *Badhamia* precedes the formation of a typical physaroid capillitium of capillary threads joined to more or less angular lime-knots. In *Badhamia gracilis,* whose capillitium is more physaroid than badhamioid, this was not found to be true.   The *Physarum*-like vacuoles and invaginations form directly without any intermediate stages.   The capillitium of *Didymium Iridis* is composed almost wholly of small tubular invaginations and any vacuoles present do not elongate, or elongate only slightly.   Harper and Dodge's description of capillitium formation in other species lends support to the belief that *Badhamia*-like vacuoles do not develop until after formation of *Physarum*-like vacuoles and tubules.

The method of capillitial formation described here and in other reports tends to discredit the theories of Zopf (**12**) and Massee (**9**) as to the manner of capillitial formation. Bisby's (**1**) statement that the capillitial threads of *Stemonitis fusca* and *Physarella mirabilis* are hollow, in conjunction with the present results, is not in accord with Zopf's theory of the formation of solid capillitial threads. The threads of *Didymium*, which have a very narrow lumen or no lumen at all at maturity, are, at first, hollow. There is no evidence that the capillitium forms from disintegrating protoplasm remaining after spore cleavage, since the capillitium is formed before spore cleavage begins. Also, it seems, all the protoplasm remaining after capillitium formation is utilized in the formation of spores during spore cleavage.

Wilson and Cadman's (**11**) report that the capillitium of *Reticularia Lycoperdon* arises by disintegration of certain tracts of protoplasm is radically different from this and previous reports. Some investigators (**7**, **8**) have referred to the capillitium of *Reticularia* as a pseudocapillitium. Perhaps this is the morphological basis for distinguishing between pseudocapillitium and true capillitium. However, since this interpretation of capillitium formation in *Reticularia* has not been confirmed, judgment must be suspended.

Strasburger (**10**) believed the capillitium to be the result of intraprotoplasmic secretion and this interpretation has been followed by Harper and Dodge (**4**) and by Howard (**5**). This might be the case in *Trichia* and *Hemitrichia*. In *Badhamia gracilis* and *Didymium Iridis*, however, this does not seem to be true. The threads arise as invaginations from the peridial wall in *Badhamia* and from the peridial wall and columella surface in *Didymium*. No sculpturing appears on the threads or lime-knots in either species. The calcareous compounds are excreted into the vacuoles in solution and the water evaporates, leaving the calcium carbonate either on the peridium or in the capillitium or both.

Kräntzlin's (**6**) interpretation of capillitial formation resulting from "elateroplasts" has been questioned by Harper and Dodge (**4**), who also attempted an explanation of her results. Since no nuclear divisions were observed in either *Badhamia* or *Didymium* until after capillitial formation, it is unlikely that any so-called elateroplasts were responsible for formation of the capillitium in either of the species.

The condensation of protoplasm, formation of capillary invaginations, and mode of spore cleavage from what are probably the relatively drier surfaces lend support to Harper and Dodge's and Cadman's hypotheses of the causes of capillitial formation and spore cleavage.

SUMMARY

The development of the capillitium of two species of Myxomycetes, *Badhamia gracilis* (Macbr.) Macbr. and *Didymium Iridis* (Ditm.) Fries, has been investigated. In *Badhamia gracilis* it was found that the capillitium develops as a result of anastomoses between tubular invaginations from the inner peridial walls and the central vacuoles resulting from protoplasmic condensation. The vacuoles and invaginations in *B. gracilis* become filled with excretory products, some of which remain as calcium carbonate after the water evaporates. The capillitium in *Didymium Iridis* also is formed by means of tubular invaginations. However, in *D. Iridis* the expanding sporangium encloses a portion of the stipe which helps make up the columella. A more or less concave area above the stipe and in the sporangium is cut off from the rest of the protoplasm. The protoplasm in this cut-off area disintegrates and the area becomes a portion of the columella. From the columella, tubular invaginations push out into the protoplasm and anastomose either with the small vacuoles which are sometimes formed there, or with the invaginations from the peridial wall, or with the peridial wall itself. Invaginations from the peridial wall sometimes connect with the columella. The small vacuoles in *Didymium* which anastomose with the invaginations are probably responsible for the nodular swellings often found on the mature capillitial threads. Calcium carbonate and other products excreted during maturation of this species are not retained in the capillitium but are transported to the exterior or to the columella.

This work was done in the mycological laboratory of the State University of Iowa under the supervision of Professor G. W. Martin.

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SOME FUNGI ON ORCHIDACEAE

Edith K. Cash and Alice J. Watson

(WITH 5 FIGURES)

During recent years a very large number of fungus specimens collected on living orchid plants by inspectors of the Plant Quarantine Branch, Agricultural Research Service, United States Department of Agriculture, at ports of entry have been referred to the writers for study and identification. Some of the fungi found have occurred only once or twice, but others have reappeared more or less frequently from year to year on interceptions made at the same or different ports of entry. It seems worth while to call attention to the more important of these fungi, particularly to those which appear to be undescribed or previously unreported on orchids.

Unfavorable conditions of shipment and storage as well as mechanical injuries due to handling are in many cases followed by the development of saprophytic fungi, so that the species present on plants when they arrive at the port of entry are frequently not those responsible for the diseased condition of the host plants. For this reason it is difficult to estimate the pathogenic importance of the organisms found. Fungi commonly found in soil or on decaying fibers, such as *Memnoniella echinata* (Riv.) L. D. Gall. and species of *Stachybotrys* and *Chaetomium*, are not infrequently present on old orchid leaves. *Gliomastix convoluta* (Harz) Mason and *Chlamydomyces palmarum* (Cke.) Mason, which are widely distributed on decaying plants of various kinds, have also been observed among such saprophytes.

In addition to describing several new species, we have commented briefly on some fungi which do not appear to fit any described species, but which have not been found in sufficient quantity or adequate condition to justify naming. Certain species are reported for the first time on orchids, and the host range of others is extended to include additional genera and species of the family.

Grateful acknowledgment is made to Dr. J. C. Lindquist and Prof. C. Cappeletti for the loan of type specimens from the Spegazzini Herbarium and the Saccardo Herbarium, respectively.

1. **Anthostomella keissleri** sp. nov. (FIG. 3)

Syn.: *Anthostomella* sp. Keissler, Beih. Bot. Centralbl. Abt. 1. **36**:
309. 1918.

Perithecia subglobosa, atra, glabra, in maculis albidis dispersa, 144–250 μ in diam., epidermide hyphis nigris nigrofacta tecta, pseudoclypeo circa ostiolum plus minusve evoluto, poro inconspicuo 10–15 μ perforata; asci cylindrici, 38–75(–110) × 3–7 μ, poro apicali praediti et interdum paraphysibus hyalinis circumdati; ascosporae olivaceo-brunneae, late fusiformes usque naviculares, inaequilaterales, 1–2-guttulatae, utrinque angustatae, oblique uniseriatae, 9–16 × 4–6 μ, plerumque 11–12 × 4–5 μ.

In caulibus foliisque Orchidearum, Mexico, America Centralis et Australis, et Insulae Philippinenses.

Perithecia subglobose, black, smooth, scattered in whitened areas on leaves and stems, thick-walled, 144–250 μ in diameter and height, partially emerging from the epidermis which is blackened by a more or less well-developed pseudo-clypeus around the ostiole, outer layer of perithecial wall made up of pseudoparenchymatic, rather thin-walled, angular cells; asci cylindrical, 38–75(–110) × 3–7 μ, with an apical pore and with or without hyaline filiform paraphyses; ascospores olivaceous-brown, inaequilateral, broad-fusiform to navicular, 1–2-guttulate, narrowed at both ends, obliquely uniseriate and slightly overlapping, 9–16 × 4–6 μ, mostly 11–12 × 4–5 μ.

On stems, leaves, and flower stalks of Orchidaceae: *Brassavola nodosa*, Canal Zone (Brownsville, Texas *63780*, Mar. 1947,[1] E. C. Harrison and R. A. Alexander); *Cattleya percivaliana*, Venezuela (San Juan, Puerto Rico, *8387*, Oct. 1942, L. J. McConnell); *Oncidium cavendishianum*, Guatemala (San Francisco, California, *24729*, Mar. 1948); *O. speciosum*, Mexico (San Francisco *10912*, TYPE, Apr. 1937); *Phalaenopsis* sp., Philippine Islands (San Francisco *25164*, June 1948, N. D. Gardner); undetermined orchid, Costa Rica (Brownsville *67112*, Nov. 1948, D. J. Smith).

Except for the unnamed species cited by Keissler (**9**, p. 309) there appear to be no species of *Anthostomella* or *Paranthostomella* described on orchids. The fungus sometimes lacks a definite, well-developed pseudoclypeus, suggesting its possible reference to *Paranthostomella;* this feature was noted by Keissler in his unnamed species on *Laelia*. However, in the genus *Paranthostomella* as described by Spegazzini (**30**, p. 42) and emended by Savulescu (**22**, p. 16) a pseudoclypeus is

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[1] The date given in citation of specimens throughout is that of the interception, and the number in parentheses is the Plant Quarantine interception number assigned to the specimen at the port of entry cited.

completely lacking. The species under study is therefore referred to *Anthostomella*.

The dimensions of asci and spores in *Anthostomella keissleri* are close to those of *A. sphaeroidea* Speg. on *Yucca* (**27**, p. 179), *A. trabutiana* Sacc. & Roum. on *Agave* (**17**, p. 27, pl. 14, f. 3) and *A. maderensis* Petr. on *Aloe* (**15**, p. 107). Since, however, specimens of these non-orchidophilous species were not available for examination, the fungus on orchids is tentatively named as new. It appears to agree with the unnamed *Anthostomella* described on *Laelia* from Mexico by Keissler, and is therefore named for him.

2. **Micropeltis bakeri** (Syd.) comb. nov.

Syn.: *Scolecopeltis bakeri* H. & P. Syd. Ann. Myc. **15**: 232. 1917.
   Scolecopeltidium bakeri (H. & P. Syd.) F. L. Stevens & Manter, Bot. Gaz. **79**: 282. 1925.

Thyriothecia superficial, hypophyllous, scattered, scutellate, dark blue-green to nearly black, composed of thin, dark, reticulately interwoven hyphae, thinner and paler toward the margin, 0.5–1 mm in diameter; ostiole circular, $20-30\,\mu$ in diameter, margin irregularly lobed or notched; asci subsessile, cylindrical-fusoid, thick-walled, 2-, 4-, or 8-spored, $90-160(-192) \times 15-20(-25)\,\mu$, rounded at the apex; ascospores hyaline, elongate-clavate or elongate-fusoid, straight or curved, 7–15-septate, $75-90(-112) \times 4-7\,\mu$, breaking up into segments; paraphyses copious, very thin-filamentous, branched, becoming agglutinated.

On leaves of *Batemannia* and *Odontoglossum chiriquense*, Costa Rica; *Cymbidium* sp., Ceylon; *Epidendrum* sp. and *Oncidium sphacelatum*, Canal Zone; ? *Lycaste* sp., *Maxillaria* sp., *Odontoglossum* sp., and *Oncidium* sp., Mexico; undetermined orchids, Brazil and Panama.

Scolecopeltis bakeri H. & P. Syd. was described from the Philippines on leaves of *Aglaia harmsiana*, *Celtis philippinensis* and *Tetrastigma sepulchrei*. No reports of its occurrence on orchids have been found. The ascospores are said to measure up to $10\,\mu$ in width, but none wider than $7\,\mu$ were observed in the exsiccati specimens examined (Baker, Fungi Malayana Nos. *586, 587*). Two-spored asci and spores up to 15-septate have also been found in the fungus on orchids; otherwise it is identical with the Philippine species.

The proper disposition of the fungus presents some difficulties. It was described by Sydow as *Scolecopeltis* and transferred by Stevens and Manter to their genus *Scolecopeltidium*, on the basis of the presence of paraphyses, and following the classification of the Dictyopelti-

neae proposed by Theissen and Sydow (**35**, p. 427–428). Both of these genera are questionably distinct from *Micropeltis,* differing according to Stevens and Manter in the filiform ascospores, which, as they point out (**33**, p. 267), is not a very satisfactory distincton. In the type species of *Scolecopeltis, S. tropicalis* Speg., the spores are reported as 15 × 4–5 μ, proportions which can scarcely be accurately described as "filiform." The breaking up of spores into segments is likewise a variable character, as is the presence or absence of paraphyses, which tend to disappear or become agglutinated into an indistinguishable mass. In view of the slight and inconstant characters on which *Scolecopeltis* and *Scolecopeltidium* were founded, both genera might well be considered to be synonyms of *Micropeltis. Scolecopeltis bakeri* is therefore transferred to this genus.

Another species differing from *M. bakeri* in its shorter ascospores has recently been noted by D. P. Limber on *Dendrobium aureum* from India (Hoboken *17358*). The blue-green scutellum is 350–400 μ in diameter; the asci are fusoid, 80–180 × 20–25 μ, surrounded by numerous filiform paraphyses; the spores are cylindrical-fusoid, 7-septate, narrowed at both ends, 37–48.4 × 7–8.5 μ. The material is inadequate in quantity for a satisfactory determination, but the characters of the fungus do not appear to agree completely with descriptions of any species of *Micropeltis, Scolecopeltis,* or *Scolecopeltidium* found.

3. **Mycosphaerella cattleyae** sp. nov. (FIG. 1)

Perithecia in maculis rotundis pallidis depressis hypophylla, brunnea, subglobosa, ostiolata, 90–150 μ in diam. et altitudine; asci numerosi, clavato-cylindrici, apice rotundati, breve pedicellati, octospori, 45–66 × 7–11 μ; ascosporae irregulariter biseriatae, fusoideae usque anguste clavatae, hyalinae, bicellulares, non constrictae, 13–17 × 2–3 μ.

In foliis Orchidearum, Guatemala, Costa Rica, Canal Zone et Mexico.

Perithecia hypophyllous, immersed, densely aggregated in pale round or elliptical sunken spots 1–3 cm in diameter or sometimes confluent, and surrounded by a heavy brown margin, usually gray above and brown beneath, dark brown, subglobose, 90–150 μ in diameter and height, with wall consisting of several layers of brown thick-walled cells and an inconspicuous ostiole; asci numerous, clavate-cylindrical, rounded at the apex and narrowed to a short pedicel, occasionally somewhat medianly constricted with four spores crowded at each end, 8-spored, 45–66 × 7–11 μ; ascospores irregularly biseriate, hyaline, fusoid to narrow-clavate, straight or curved, acute at the ends, 4-guttulate to 1-septate, not constricted, 13–17 × 2–3 μ; paraphysoids subhyaline.

On leaves of Orchidaceae: *Cattleya aurantiaca,* Guatemala (San Francisco, California, *16732,* Jan. 1940, W. H. Wheeler) ; *C. skinneri,*
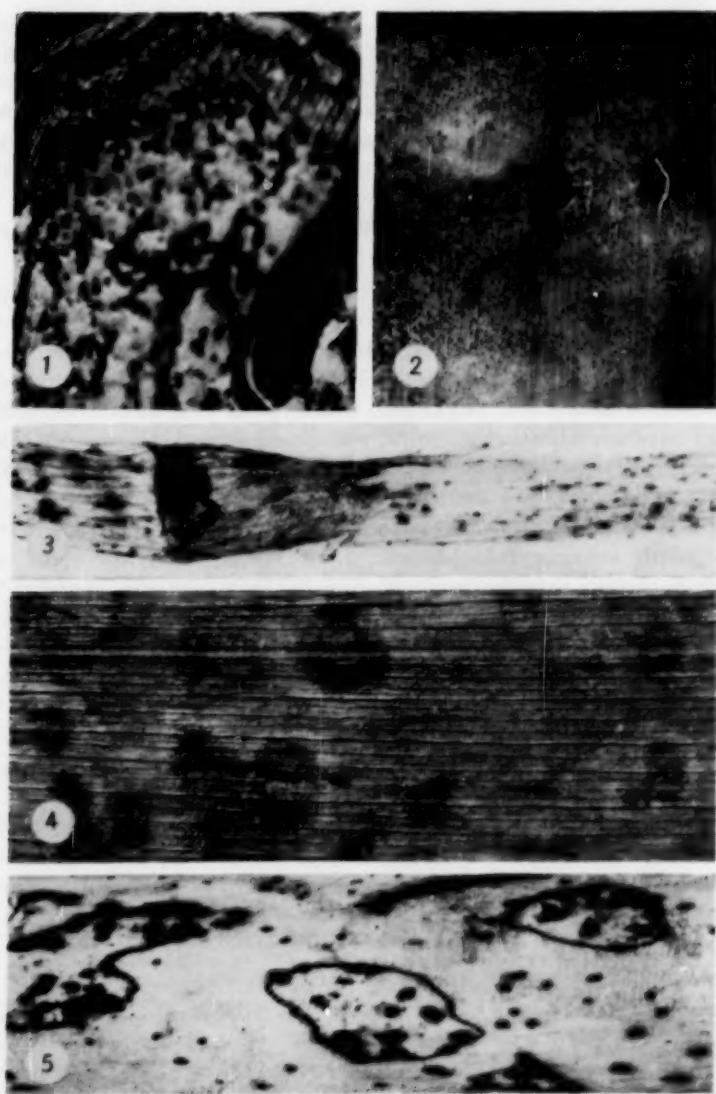
FIG. 1. *Mycosphaerella cattleyae* on *Epidendrum radicans*, Mexico, × 10. 2. *Phyllostictina pyriformis* on *Schomburgkia*, Grand Cayman Island, Brit. West Indies, × 2. 3. *Anthostomella keissleri* on *Brassavola nodosa*, Canal Zone, × 5. 4. *Septoria selenophomoides* on *Cymbidium devonianum*, India, × 2. 5. *Phomopsis orchidophila* on *Oncidium splendidum*, Guatemala, × 5. (Fig. 1, phot. A. Lohr; Figs. 2–5, R. Taylor.)

Guatemala (San Francisco *17183*, S௷. 1940, W. H. Wheeler), and
Costa Rica (Hoboken, New Jersey, *1834*, June 1941, D. P. Limber);
on *C.* sp., Canal Zone (Laredo, Texas, *36811*, TYPE, June 1945, J. B. R.
Leary); *Epidendrum radicans*, Costa Rica (Miami, Florida, *1309*, June
1939, A. S. Mills), and Mexico (San Francisco *15004*, May 1938, W. H.
Wheeler); on *Laelia* sp., Guatemala (Brownsville, Texas, *62637*, Oct.
1946, D. J. Smith).

The narrow spores distinguish the species from others of the genus
found on Orchidaceae.

A species of *Mycosphaerella* with biconic ascospores has been ob-
served several times on intercepted orchid specimens: *Miltonia vexillaria*
from Colombia, *Cymbidium elegans* from India and an undetermined
orchid from Brazil. The perithecia of this fungus are hypophyllous,
evenly distributed over large spots which are at first brown, becoming
paler to nearly white in the center, with a well-defined border, 150–175 μ
in diameter; asci subglobose at first, then clavate, 50–57 × 13–15 μ,
abruptly narrowed to a short pedicel, broadly rounded to slightly obtuse
at the apex, rarely curved; ascospores hyaline, bicellular, not constricted,
irregularly biseriate, fusoid-biconic, 15–17.5(–20) × 5 μ, the upper cell
slightly broader than the lower, surrounded by a gelatinous sheath 0.5 μ
thick. More adequate material is needed for identification of this fungus.

Other fungi apparently belonging to *Mycosphaerella* and different
from the two species discussed above have been found on orchids, but
in each instance only one rather poor specimen has been examined. A
species with fusoid asci, 70–75 × 12–15 μ, and large, subhyaline, curved
spores, 20–28 × 4–6 μ, was observed on *Odontoglossum grande* inter-
cepted from Guatemala. · Another *Mycosphaerella* on a specimen of
Cymbidium giganteum from India we have tentatively referred to *M.
calceoli* Kirschstein & Kirulis, described (**11**, p. 210) from Latvia on
Cypripedium calceolus, a species characterized by its small asci and
spores.

4. **Ophiodothella orchidearum** sp. nov.

Perithecia in maculis pallidis dispersa, subepidermalia, membranacea, atro-
brunnea, lenticularia usque depresso-globosa, 300–500 μ in diam., 200–300 μ alta,
ostiolo brevi, pseudoclypeo nigro circumdata et poro 25 μ in diam. praedito; asci
arcuati vel recti, cylindrico-fusoidei, stipitem brevem versus et ad apicem angus-
tiorem rotundatum attenuati, octospori, 65–95 × 6–9 μ; ascosporae elongatae, rectae
vel spiriliter tortae, multiguttulatae usque 3–12-septatae, hyalinae usque viridius-
culae, 55–66 × 1.8–3 μ; paraphyses filiformes, flexuosae, guttulatae, apice 1–1.5 μ.

In caulibus, scapis et pseudobulbis Orchidearum, Venezuela, Colombia, Guate-
mala, Brasilia, Insulae Philippinenses et Mexico.

Perithecia scattered over pale, dark-margined spots on undefined whitened areas of leaves and stems, subepidermal, dark brown, membranaceous, lenticular to depressed-globose, 300–500 μ in diameter, 200–300 μ high; with a short, inconspicuous ostiole and pore 25 μ in diameter, the blackened host tissue surrounding the ostiole forming a pseudoclypeus; asci arcuate or straight, cylindrical-fusoid, abruptly narrowed to a short stipe and toward the narrow, rounded apex, 8-spored, 65–95 × 6–9 μ; ascospores elongate, straight or spirally twisted in the ascus, multiguttulate to 3–12-septate, often slightly constricted about one-third the length from each end, hyaline to pale greenish, 55–66 × 1.8–3 μ; paraphyses filiform, flexuous, guttulate, hyaline, 1–1.5 μ.

On stems, flower stalks, flower scapes, and pseudobulbs of Orchidaceae: *Cattleya mossiae*, Venezuela; *C.* sp., Venezuela and Colombia; *Laelia superbiens*, Guatemala (San Francisco *18940*, TYPE, Feb. 1945, W. H. Wheeler); *Laelia* sp., Brazil; *Odontoglossum uroskinneri*, Guatemala; *Oncidium bicallosum*, *O. cavendishianum*, *O. leucochilum*, *O. splendidum*, Guatemala; *Phalaenopsis schilleriana*, Philippine Islands; *Sobralia xantholeuca*, Mexico; *S.* sp., Guatemala; undetermined orchid, Mexico.

Dark hyphae invading the host tissue around the ostiole form a more or less conspicuous pseudoclypeus, and for this reason the fungus has been assigned to *Ophiodothella*, rather than *Ophiobolus*. Since *Linospora subtropicalis* Speg. appeared from the description (**28**, p. 403) to be similar, the *Ophiodothella* was compared with type material (Spegazzini Collecciones Micologicas *884*, on *Oncidium*, Salta San Lorenzo, Apr. 2, 1905) examined through the courtesy of Dr. J. C. Lindquist. No asci or spores could be found in the microscopic mount made from the type of *L. subtropicalis;* however, the superficial perithecia, devoid of any suggestion of a clypeus, provide sufficient evidence that it is a different species.

5. **Paranthostomella microspora** sp. nov.

Perithecia atra, subglobosa, membranacea, in maculis albis atromarginatis dispersis, ostiolo leniter protruso, clypeo carenti, 112–150(–270) μ in diam.; asci cylindrici, 34–60 × 3–4 μ, paraphysibus filamentosis hyalinis circumdati; ascosporae viridulae, dein olivaceobrunneae, ellipsoideae usque naviculares, oblique uniseriatae, 5–6(–8) × 2–3(–4) μ.

In foliis caulibusque Orchidearum, Venezuela, Guatemala et Mexico.

Perithecia black, subglobose, scattered in white, black-bordered spots on leaves and stems, immersed, with ostiole slightly protruding, wall 15–20 μ thick, composed of dark brown pseudoparenchyma, 112–150 (–270) μ in diameter; asci cylindrical with apical pore, 34–60 × 3–4 μ;

ascospores pale greenish at first, becoming olivaceous brown, ellipsoid to navicular, inaequilateral, with obtuse ends, obliquely uniseriate, 5-6(-8) × 2-3(-4) μ; paraphyses filiform, hyaline.

On stems and leaves of Orchidaceae: *Cattleya lueddemanniana*, Venezuela (San Juan, Puerto Rico, *8127*, Jan. 1942, L. J. McConnell); *Laelia anceps*, Mexico (Brownsville, Texas, *64903*, TYPE, July 1947); *Laelia* sp., Mexico (Brownsville *48130*, June 1941, E. P. Reagan *et al.*); *Oncidium wentworthianum*, Guatemala (Brownsville *63727*, Mar. 1947, D. J. Smith).

The fungus differs from most species of the genus in the small asci and spores and from *Anthostomella bromeliae* Starb. (**32**, p. 61) in the presence of paraphyses.

6. Physalospora

The species of this genus found on intercepted orchids fall into three convenient groups on the basis of the dimensions of the ascospores: (1) those with spores less than 20 μ in length, which we have referred to *Physalospora wildemanniana* Sacc.; (2) those with ascospores between 20 μ and 25 μ long, to *P. camptospora* Sacc.; and (3) one specimen with very long ascospores (25-29 × 3.4-4 μ) on *Laelia* from Guatemala (San Francisco *20778*).

We have had no opportunity to examine type material of the species of *Physalospora* described on orchids, but judging from the descriptions and available specimens, *P. cattleyae* Maubl. & Lasnier (**13**, p. 171, pl. 12) does not appear to differ from *P. camptospora* Sacc. (**18**, p. 127, pl. 3, f. 1); as described, *P. orchidearum* P. Henn. (**5**, p. 170) cannot be distinguished from *P. wildemanniana* Sacc. (**18**, p. 128, pl. 3, f. 2). The names having priority of publication have therefore been used.

The fungus referred by us to *Physalospora camptospora* Sacc. has been found on species of *Cattleya* from Guatemala, *Coelogyne* from India, *Dendrobium* from Australia, *Oncidium* from Colombia, and *Laelia* and *Stanhopea* from Mexico. Specimens which we have determined as *P. wildemanniana* Sacc. are on hosts of the following genera: *Cattleya*, *Cymbidium*, *Dendrobium*, *Epidendrum*, *Laelia*, *Lycaste*, *Oncidium*, *Phalaenopsis*, *Stanhopea*, *Trichopilia*, and *Vanda;* the localities include Mexico, Guatemala, Trinidad, Dominican Republic, Colombia, Venezuela, India, Siam and the Philippine Islands.

7. **Valsella pedicellata** sp. nov.

Perithecia in stromatibus valsoideis, 0.4-0.5 mm in diam., in areis albis nigro-marginatis aggregata, singula vel 2-3 in stromate quoque caespitosa, 250-300 μ in

diam., subglobosa, atra, parietibus nigris carbonaceis, plectenchymaticis, 40–50 μ crassis, ostiolis brevibus, crassis, integris, periphysibus hyalinis intus vestitis, in discum subplanum primo album dein hyphis nigris tectum emergentibus; asci clavati usque clavato-cylindrici, interdum subsessiles vel saepius longe pedicellati, polyspori, 75–100 (p. sp. 50–60) × 12–15 μ; ascosporae subhyalinae, in massa brunneae, allantoideae, 4–6(–8) × 0.7–1 μ; paraphyses non visae.

In foliis caulibusque Orchidearum, Guatemala, Mexico, et Siam.

Stromata valsoid, black, scattered over whitened areas of stems and leaves, 0.4–0.5 mm in diameter and height, bordered by a black line evident on the surface; perithecia solitary or 2–3 to each stroma, 250–300 μ in diameter, subglobose, walls black, carbonaceous, plectenchymatic, ostioles short, thick, entire, lined with hyaline filamentous periphyses, emerging on a more or less flattened disk which is white when exposed but more often covered by black hyphae; asci clavate to clavate-cylindrical, sometimes subsessile but often long-pedicellate, myriosporous, 75–100 (p. sp. 50–60) × 12–15 μ; ascospores subhyaline, brownish in mass, allantoid, 4–6(–8) × 0.7–1 μ; paraphyses not seen.

On leaves and stems of Orchidaceae: *Odontoglossum* sp., Mexico (Laredo, Texas *38343*, Feb. 1946, T. P. Chapman); *Oncidium ampliatum*, Canal Zone (San Francisco, California *30036*, E. V. Lehner & F. M. Thompson, Feb. 1953); *O. cavendishianum*, Guatemala (San Francisco *23040*, Mar. 1947, F. L. Blanc); *O. splendidum*, Guatemala (San Francisco *18981*, TYPE, Mar. 1945, W. H. Wheeler); *Vanda coerulea*, Siam (San Francisco *24925*, Apr. 1948, N. D. Gardner); undetermined orchid, Mexico (Laredo *41680*, Nov. 1946, R. M. Fouts).

No species of *Valsella* has been described on orchids. The inconspicuous stromatic development and pedicellate asci suggest *Cryptosphaerella*, but the delimiting black line surrounding the valsoid stroma seems to place the fungus in *Valsella* rather than in the former genus. Although Berlese characterizes the asci in *Valsella* as sessile, he describes and illustrates two species, *V. amphoraria* (Nits.) Sacc. var. *padina* Sacc. and *V. alnicola* Berl. (2, p. 107, pl. 133) with pedicellate asci.

8. **Phyllostictina pyriformis** sp. nov. (FIG. 2)

Pycnidia amphigena, saepius hypophylla, in maculis magnis pallidis circularibus vel ellipsoideis aequaliter et subdense vel interdum super superficiem totam folii dispersa, depresso-globosa, 150–200 μ in diam., ostiolo prominenti 10–15 μ diam. pertusa, pariete e cellulis magnis irregularibus crasse tunicatis composito, viridofusco vel fere nigro, matricem gelatinosam sporogenam circumdanti; sporidia primum histogenice e pariete nata, dein ex apicibus conidiophororum cylindricorum usque subuliformium 1-2-septatorum, simplicium, 10–15 × 1–1.5 μ, singulatim oriundia, late ellipsoidea usque subpyriformia vel irregularia, granulosa, saepe uni-

guttulata, 9–12 × 5–7(–10) μ, conidiophoro persistenti et saepe apice appendicula brevi filiformi praedita.

In foliis caulibusque Orchidearum, Mexico, America Centralis et Australis, Insulae Atlanticae et Pacificae, India et Australia.

Pycnidia amphigenous, more frequently hypophyllous, evenly but rather densely scattered over large circular to ellipsoid pallid spots, sometimes distributed over most of the leaf surface, depressed-globose, 150–200 μ in diameter, with prominent ostiole 10–15 μ in diameter; pycnidial wall made up of large irregular thick-walled cells, dark greenish-brown to nearly black, enclosing a gelatinous matrix from which the first spores develop histogenically; conidiophores later developed, cylindrical to subulate, 1–2-septate, unbranched, 10–15 × 1–1.5 μ; spores broadly ellipsoid to subpyriform or irregular, granulose, frequently with one large guttule, 9–12 × 5–7(–10) μ, with the conidiophore often remaining attached and frequently having a filiform appendage at the apical end.

On leaves and stems of Orchidaceae: *Arundina bambusifolia*, Costa Rica; *Cattleya skinneri*, Guatemala; *Coelogyne* sp., Philippine Islands; *Cypripedium insigne*, India; *Cypripedium* sp., Japan; *Dendrobium canaliculatum*, Australia; *D. phalaenopsis*, Australia and Philippine Islands; *D. schuetzei*, Philippine Islands; *D. undulatum*, Australia; *D.* sp., Australia, Samoa, and Haiti; *Epidendrum fragrans*, Jamaica; *E.* sp., Mexico; *Jacquiniella* sp., Canal Zone; *Laelia anceps*, Mexico; *Oncidium*, Canal Zone; *Schomburgkia tibicinis*, Mexico; *S.* sp., Grand Cayman Island, British West Indies (Hoboken, New Jersey *4249*, TYPE, May 1945, H. L. Sanford); *Stanhopea* sp., Costa Rica; and undetermined orchids, Honduras and Canal Zone.

In view of the histolysis of the pycnidial parenchyma and the appendiculate spores, we are tentatively placing this fungus in *Phyllostictina*, although with some hesitation, since conceptions of the genus held by various authors differ. Our fungus agrees with the characters described for *Phoma citricarpa* McAlp., the pycnidial stage of *Guignardia citricarpa* Kiely, as described by Darnell-Smith (3) and Kiely (10). According to Kiely (10, p. 260), Shear expressed the opinion that *Phoma citricarpa* should be placed in *Phyllostictina*, but the formal transfer to that genus was not made until 1953 by Petrak (16, p. 115).

Phyllostictina murrayae Syd., the type of the genus, was described by Sydow (34, p. 185–186) on leaves of *Murraya koenigii* from India. Neither Sydow nor Hoehnel, who later studied the type and emended the genus (6), found conidiophores, the latter author assuming that they had been present but had disappeared. In *P. pyriformis* conidiophores are present in some material examined, while in other cases the spores

are produced by histolysis. A transformation of the pycnidial wall from pseudo-parenchyma to spore-bearing hyphae, similar to the process described and illustrated by Darnell-Smith (**3**, pl. 89) for *Phyllostictina citricarpa* (McAlp.) Petr., presumably takes place in *P. pyriformis*.

The gelatinous cap on the apical end of the pycnospore, which later contracts and becomes a filiform appendage, is also variable in *P. pyriformis*, being present in some cases, while no trace of it can be observed in others. These appendages have been reported by Darnell-Smith (**3**) and Kiely (**10**) in *Phyllostictina citricarpa*, by Shear (**24**, p. 316, and **25**, p. 14–15) in *P. vaccinii* Shear, and by Guba (**4**) in *Phyllosticta solitaria* Ell. & Ev.

Shear (**26**) restricts the form-genus *Phyllostictina* to the pycnidial stages of *Guignardia*. An immature ascomycete which is present occasionally associated with *Phyllostictina pyriformis* on orchid leaves has never been found in sufficient quantity or mature enough condition to afford even a guess as to its generic position. This may possibly be one of the two species of *Guignardia* reported on the Orchidaceae, namely *G. microsticta* Sacc. (**21**, p. 75) on *Cattleya* from France or *G. traversi* (Cav.) Sacc. & Trott. (**21**) (*Laestadia traversi* Cav. F. Longob. Exsicc. *231*), found on *Vanilla planifolia* in Italy.

Two species of *Phyllosticta* described on orchids appear to resemble *Phyllostictina pyriformis* in the size and shape of the pycnospores: *Phyllosticta cymbidii* Sawada on *Cymbidium aloifolium* from Formosa (**23**, p. 62, pl. 3, f. 18) and *P. phalaenopsidis* Marchal & Verplancke (**12**, p. 22, fig. 5), but no material of either has been available for examination, and from the brief descriptions (the former in Japanese), it was not possible to identify our fungus definitely with either one of these species.

9. **Phomopsis orchidophila** sp. nov. (FIG. 5)

Pycnidia in maculis pallidis linea atra angusta marginatis dispersa, immersa, denique erumpentia, atra, stromatica, lenticularia, 100–200 µ in diam., 75–100 µ alta, e textura atro-brunnea ad basim pallidiore, ad apicem obscuriore composita, e conidiophoris subuliformibus 10–15 × 2–3 µ vestita; conidia bigeneria, ambobus interdum in pycnidio singulo praesentibus: (A) ellipsoideo-fusoidea apicibus sub-acutis, hyalina, 2-guttulata, 4–8 × 1.5–3 µ; (B) filiformia, hyalina, hamata, 16–30 × 0.5–1 µ.

In caulibus, foliis vaginisque Orchidearum, Mexico, Guatemala, Puerto Rico, Colombia, Venezuela, India, Australia et Insulae Pacificae.

Pycnidia scattered over pale spots usually margined by a thin black line, immersed then erumpent, black, stromatic, lenticular, 100–200 µ in

diameter, 75–100 μ high, pseudoparenchymatic, paler at the base, dark brown and thicker above, lined with subuliform conidiophores 10–15 × 2–3 μ; A-spores ellipsoid-fusoid, with subacute ends, hyaline, 2-guttulate, 4–8 × 1.5–3 μ; B-spores filiform, hyaline, hamate, 16–30 × 0.5–1 μ, both kinds sometimes present in a single pycnidium.

On stems, leaves, and flower-sheaths of Orchidaceae: *Catasetum viridiflavum*, Canal Zone; *Cattleya gigas*, Colombia; *C. mossiae*, Venezuela; *C.* sp., Colombia; *Coelogyne barbata*, India; *Cymbidium aloifolium*, India; *Dendrobium atroviolaceum*, Australia; *D.* sp., Solomon Islands; *Epidendrum atropurpureum*, Mexico; *Laelia anceps* and *L. gouldiana*, Mexico; *Oncidium crispum*, Guatemala; *O. splendidum*, Guatemala (San Francisco *23417*, TYPE, Apr. 1947); *O. tigrinum*, Mexico; *O. wentworthianum*, Guatemala; *Stanhopea* (?), Guatemala; *Vanda coerulea*, India; *V. sanderiana*, Philippine Islands, and undetermined orchid, Trinidad.

It is possible that this *Phomopsis* is synonymous with *P. calanthes* Sacc., the only species of the genus found reported on orchids, but its identity with this species could not be established from the brief description (**20**, p. 209). The A-spores of the two fungi are very similar in size and shape; no measurements of B-spores of *P. calanthes* are given by Saccardo. Type material of the latter species from the Saccardo herbarium, loaned to us for comparison, through the courtesy of Prof. C. Cappeletti, showed only empty pycnidia.

10. **Septoria selenophomoides** sp. nov. (FIG. 4)

Pycnidia in maculis circularibus olivaceo-viridulis usque bubalinis indefinitis aggregata, amphigena, saepius hypophylla, subepidermalia dein erumpentia, brunnea, pseudoparenchymatica, primum parvo-ostiolata, in maturitate late aperta, pariete cellulis cubicis vel ovoideis composito, 86–300 μ in diam.; conidiophora hyalina, subulata, 4–7 × 1–2 μ; conidia singulatim oriunda, hyalina, bigeneria; alia brevia, continua, curvata, 5–6 × 1 μ, alia longiora, filiformia, continua vel uniseptata, recta curvatave, rare sigmoidea, extremos obtusos versus attenuata, 12–22 × 0.7–1 μ, plerumque 12–15 × 1 μ, in cirrhis albidis emergentia.

In foliis Orchidearum, Mexico, Guatemala, Brasilia, Colombia, Anglia, India, et Japonia.

Pycnidia aggregated in circular olive-greenish to buff spots without well-defined border, visible on both leaf surfaces, amphigenous but more often hypophyllous, subepidermal, then erumpent, brown, pseudoparenchymatic, at first with a small circular ostiole, later opening widely at maturity, wall formed of several layers of cubical to ovoid cells from which the conidiophores develop, 86–300 μ in diameter; conidiophores

hyaline, subulate, $4-7 \times 1-2 \mu$; conidia hyaline; filiform, borne singly, of two types: microconidia short, continuous, curved, $5-6 \times 1 \mu$; macroconidia longer, filiform, continuous or uniseptate, straight or curved, rarely sigmoid, narrowed toward the obtuse ends, $12-22 \times 0.7-1 \mu$, usually $12-15 \times 1 \mu$, emerging in white cirrhi.

On leaves of Orchidaceae: *Brassia verrucosa*, Guatemala; *Bulbophyllum mapellii*, Brazil; *B.* sp., India; *Cattleya citrina*, Mexico; *Coelogyne cristata*, India; *C. elata*, India; *Cymbidium devonianum*, *C. pendulum*, *C. whitiae*, India; *C.* sp., England; *Cypripedium insigne* and *C.* sp., India; *Dendrobium ashworthiae*, England; *D. nobile*, Brazil; *D. phalaenopsis schroederianum*, England; *D.* sp., Japan; *Epidendrum cochleatum* and *E. fragrans*, Guatemala; *Laelia tenebrosa* and *L.* sp., Brazil; *Masdevallia ignea*, England; *Miltonia regnelii*, Brazil; *Odontoglossum bictoniense*, Guatemala; *O. citrosum*, Mexico; *O. cordatum*, Guatemala; *O. uroskinneri*, Guatemala; *O.* sp., Mexico (Laredo *51860*, TYPE, Nov. 1951, A. H. Lewis); *Oncidium rogersii* and *O. speciosum*, Brazil; *O. wentworthianum*, Guatemala; *O.* sp., Guatemala and Brazil; *Stanhopea* sp., Mexico; undetermined orchids, Mexico, Guatemala, and Colombia.

Many of the collections listed above were determined by us during past years under other names, the two spore-forms having been considered as separate species. We sometimes labeled specimens in the microspore stage *Selenophoma* or *Phyllosticta;* the structure of the pycnidia and the curved spores are strongly suggestive of the former genus. The macrospore condition we have frequently determined as *Septoria posekensis* Sacc., to which it shows close resemblance. After sufficient material had been examined, it eventually became clear that the two forms belong to a single fungus, both stages being present in some instances in the same pycnidium. Both short and long spores developed in culture. In the structure of pycnidia and in the conidiophores and two types of pycnospores the fungus greatly resembles Sprague's illustrations of some of the species of *Septoria* on grasses (**31**, pp. 238, 240, 248).

Cultures of this fungus from a specimen of *Odontoglossum* from Mexico (Laredo *51860*) were made on potato dextrose agar. Pale gray, very restricted aerial mycelial growth developed and pycnidia and spores were produced. Both short and long spores were formed, the latter simple or occasionally one-septate, with rather blunt ends, and slightly longer and broader than those found in nature.

Among the descriptions of species of *Septoria* on orchids, our fungus most nearly resembles that of *S. posekensis* Sacc., found on an undeter-

mined orchid from Siberia (**19**, p. 274). For that reason the fungus found on many genera of orchids intercepted from various localities was for some years referred to that species. The thin-walled pycnidia, densely aggregated on greenish olivaceous spots, and relatively short, very slender spores are characters agreeing with those of *S. posekensis,* from which *S. selenophomoides* differs chiefly in the larger, more widely opened and hypophyllous pycnidia, and the occurrence of microconidia. Type material of Saccardo's fungus was not available at the Saccardo Herbarium, so that a comparison with it was impossible.

Septoria thelymitrae McAlpine on *Thelymitra aristata* in Australia (**14**, p. 101) has broader spores and smaller, black pycnidia growing on whitish, dark-margined spots. *S. orchidearum* West. (**37**, p. 393) differs in pointed spores. All of the other species of *Septoria* reported on the Orchidaceae have longer spores.

It is possible that *Phyllosticta nigro-maculans* Sacc., described on an unnamed orchid from Brazil (**18**, p. 129, pl. 4, f. 5), may be identical with the microspore stage of *Septoria selenophomoides.* The short, curved spores are close in shape and dimensions. The black mycelium described by Saccardo is, however, absent from our specimens.

Since type material of neither of Saccardo's species, *Phyllosticta nigro-maculans* and *Septoria posekensis,* could be obtained for comparison, it has seemed advisable to describe the fungus we have found on intercepted orchid material as a new species, which we have named *S. selenophomoides* because of the resemblance of the microspores to those of that genus.

11. **Camarosporium orchidicola** sp. nov.

Pycnidia in areis albidis linea atra definitis dispersa, pseudoparenchymatica, atra, erumpentia, depresso-globosa usque subconica, 100–300 µ in diam., 100–150 µ alta, apice irregulariter dehiscentia; conidiophora inconspicua, hyalinia, cylindrica, 10 × 1 µ; sporae rubro-brunneae, aliae subglobosae, in medio uniseptatae, cellulis longitudine interdum irregulariter radiatim vel cruciatim septatis, 5–8(–11) × 5–6(–8) µ, aliae oblongae vel ovatae, horizontaliter 3-septatae, cellulis centralibus longitudine septatis, non constrictae, 9–14(–18) × 6–9 µ.

In caulibus foliisque Orchidearum, Mexico, Costa Rica, Canal Zone, Honduras et Australia.

Pycnidia scattered over whitened areas of stems and leaves, surrounded by narrow black lines, black, pseudoparenchymatous, erumpent, depressed-globose to subconical, 100–300 × 100–150 µ, breaking open irregularly, narrowed toward the apex but not acute; conidiophores indistinct, hyaline, cylindrical, simple, 10 × 1 µ; spores reddish-brown,

some globose with one horizontal septum near the center and one or rarely both cells longitudinally septate, or irregularly cruciately or radially divided, 5–8(–11) × 5–6(–8) μ, other spores oblong or ovate with 3 horizontal septa and center cells longitudinally septate, not constricted or rarely slightly so, 9–14(–18) × 6–9 μ.

On stems and leaves of Orchidaceae: *Arundina* (*?bambusifolia*), Costa Rica; *Brassavola nodosa*, Canal Zone; *Cymbidium canaliculatum*, Australia; *Epidendrum* sp., Mexico; *Laelia gouldiana* and *L. majalis*, Mexico; *Odontoglossum insleayi*, Mexico; *Oncidium* sp., Honduras (San Francisco *6980,* TYPE, Feb. 1936, R. D. Clemens); undetermined orchid, Mexico.

No reports of *Camarosporium* occurring on orchids have been found. The spores of *C. orchidicola* agree in dimensions with those of *C. aequivocum* (Pass.) Sacc. but differ in the reddish brown color. The latter species has been reported only on *Artemisia* and *Tanacetum* in Europe.

12. GLOEOSPORIUM and COLLETOTRICHUM

Verplancke (**36**) listed 35 species of *Gloeosporium* and *Colletotrichum* on the Orchidaceae and others have been described in the twenty years since his tabulation was made. Many of these species apparently were named chiefly on a host basis, from their occurrence on certain genera of orchids, and cannot be differentiated by morphological characters such as dimensions of acervuli, setae or spores. For example, Verplancke's list includes 20 species of the general type of *Colletotrichum orchidearum* which differ to some degree in the size of the acervuli and length of conidiophores, but in all of which the spores range from 14 to 20 μ in length and 4 to 6 μ in width. It seems highly improbable that these are all distinct species, and a few have already been reduced to synonymy by various authors. However, much investigation will be necessary, including examination of type specimens where available and cross inoculations among various orchid genera, before the nomenclature of these fungi is on a stable basis.

Some species of *Colletotrichum* and *Gloeosporium* on orchids are undoubtedly pathogens, as is the case with species of these genera causing diseases in other host plants, but it is evident that in much of the material examined, intercepted from imported orchids, the fungi have developed saprophytically on plants injured by other organisms or weakened by unfavorable conditions of shipment. It is obviously impossible to estimate the pathogenic importance of the fungi concerned from the examination of a few leaves collected at the port of entry and

sent in for determination. Until further taxonomic study of the species
of these genera found on orchids has been made, it does not seem
advisable to attempt specific determinations.

13. **Septonema intercalare** sp. nov.

Conidiorum catenae dense compactae massas atras pulverulentas in superficie
matricis efformantes, e 2–3 cellulis basalibus subglobosis, toruloideis, viridi-brunneis
2–3 μ in diam. oriundae, 150–175 μ longae, simplices vel 2–3-furcatae, e conidiis et
cellulis parvis intercalaribus compositae, demum rumpentes; conidia acropetale
evoluta, crasse tunicata, oblongo-ellipsoidea, pallide olivacea denique fusco-brunnea,
unicellularia usque 5-septata, 11–25 × 4–6 μ, plerumque 12–14 × 5–6 μ (cellulis
intercalaribus exclusis); cellulae intercalares 1–2, globosae, 2–3 μ in diam., subhya-
linae, ad apicem conidii oriundae et persistentes.

In foliis, caulibus, et pseudobulbis Orchidearum, Mexico, Costa Rica, Canal
Zone, et Venezuela.

Closely compact chains of cells forming pulverulent masses on the
host surface; no well-developed conidiophores, the conidial chains origi-
nating from 2–3 toruloid, subglobose, pale greenish-brown basal cells
2–3 μ in diameter; conidial filaments 150–175 μ long, simple or 2–3-
branched; conidia thick-walled, oblong-ellipsoid, at first pale olivaceous,
becoming dark brown, simple to 5-septate, developed acropetally, 11–25
× 4–6 μ, mostly 12–14 × 5–6 μ, excluding the intercalary cells; inter-
calary cells 1–2, globose, 2–3 μ in diameter, hyaline to subhyaline, devel-
oping at the distal cell of each conidium and remaining attached to the
conidium after the chain breaks up; branching of chain occurring where
two intercalary cells are formed on a single conidium and a new
conidium is developed from each.

On leaves, stems and pseudobulbs of Orchidaceae: *Cattleya bow-
ringiana*, Canal Zone (San Francisco, California, *26493*, Nov. 1949,
A. S. Johnson); *Laelia* sp., Venezuela (Brownsville, Texas, *62939*,
TYPE, Oct. 1946, R. A. Alexander); *Odontoglossum* sp., Costa Rica
(San Francisco *27438*, Nov. 1950, L. J. Lefebvre and F. M. Thomp-
son); *Oncidium sphacelatum*, Mexico (El Paso, Texas, *51249*, Aug.
1948, E. Smith).

Septonema intercalare appears to be distinct from other species of
the genus in the presence of intercalary cells separating the conidia and
persisting at the apical end of each conidium after the chains have fallen
apart. In *S. toruloides* Berl. (**1**, p. 103, pl. 10, f. 18–20) and in *Torula
herbarum* (Hughes **7**, p. 635) the differentiated cells present are of the
same diameter as the other cells of the conidia, differing in their darker
color and roughened walls, while in *S. intercalare* the connecting cells
are much smaller smooth and hyaline.

14. SEPTONEMA ORCHIDOPHILUM Speg. var. **longisporum** var. nov.

Caespituli parvi dispersi vel areas atras pulverulentas 1–2 mm diam. efficientes; conidiophori inconspicui, breves, pallide viridi-brunnei, septati, 10–15×2–$3\,\mu$; conidia in catenis simplicibus vel ramosis mox rumpentibus acropetale evoluta, cylindrica, apicibus rotundata vel subobtusa, 10–$20(-26) \times 4$–$5(-6)\,\mu$, 1–6-septata, pallide olivacea dein brunnea, ad septa leniter constricta. A varietate typica conidiis longioribus differt.

In foliis et vaginis floralibus Orchidearum, Guatemala, Costa Rica, et Mexico.

Forming small punctiform tufts or larger effuse black pulverulent patches 1–2 mm in diameter; conidiophores scarcely evident, short, pale greenish-brown, septate, 10–15×2–$3\,\mu$; conidia in simple or rarely branched chains which break up readily, cylindrical-ellipsoid, rounded or slightly obtuse at the ends, 10–$20(-26) \times 4$–$5(-6)\,\mu$, 1–6-septate, somewhat constricted at the septa, pale olivaceous at first, becoming dark brown, developing acropetally by the budding of the distal cell of the terminal spore, the lateral chains similarly produced from lateral budding, the basal cell of the chain being the first formed.

On leaves and flower sheaths of Orchidaceae: *Cattleya dowiana,* Costa Rica (San Francisco, California, *25406,* Nov. 1948, A. S. Johnson); *C. skinneri,* Guatemala (Brownsville, Texas, *66088,* Apr. 1948) and Costa Rica (San Francisco *18446,* Dec. 1943, W. H. Wheeler); *Oncidium* sp., Mexico (San Francisco *22631,* TYPE, Jan. 3, 1949, A. S. Johnson).

Except for the longer spores, the fungus agrees in essential features with the description of *Septonema orchidophilum* Speg. (**29,** p. 438), described on *Oncidium* in Argentina, and is therefore tentatively referred to that species as a variety.

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TWO NEW SPECIES OF CHAETOMIUM AND ONE NEW HUMICOLA SPECIES

Aasa Omvik

(WITH 5 FIGURES)

The effect of manganese, zinc and calcium in nutrient solutions for a group of fungi isolated from Norwegian soils has previously been investigated by the author (9). In the group of fungi treated were included two species of *Chaetomium* and one *Humicola* species which, to the knowledge of the author, have not been described before. Investigations concerning the role of zinc as a minor element for fungi will be carried on by the author. Zinc proved to be a valuable growth factor for the three species mentioned, as for most of the fungi treated. Therefore, a description of the two species of *Chaetomium* and the *Humicola* species not previously described is considered to be necessary.

In 1948, Skolko and Groves (11) described and illustrated *Chaetomium* species with dichotomously branched perithecial hairs; recently the same authors (12) have presented thorough descriptions and figures of the remaining species of the genus. The new species (about 30) which have appeared since Chivers (3) monographed the genus *Chaetomium* were treated, and the species which have already been invalidated through synonymy were referred to the names under which they were first described, with the result that 47 species have been retained as valid. The two papers of Skolko and Groves represent an excellent tool in identifying *Chaetomium* species. The descriptions and figures given in these papers have been minutely studied by the author. Further, a culture received from the Centraalbureau voor Schimmelcultures, Baarn, under the name of *Chaetomium tortuosum* Garbowski, has been examined, this species not having been described by Skolko and Groves. These authors also exclude from description the three following species of *Chaetomium* described as new by Ames (2): *C. fibripilium*, *C. mollipilium,* and *C. nigricolor,* and, therefore, the descriptions presented by Ames (2) have been studied. Further, the monograph of Chivers (3) and the papers describing the following species of *Chaetomium* have been consulted: *C. fiscicolum* Petrak (10) (according to C. J. Hughes a species of *Sordaria* (12, p. 807)); *C. subterraneum* (syn. *C. globosum*

(15)) Swift and Povah (13); *C. minimum* (syn. *C. aureum* (12, p. 786)) Van Beyma (16); *C. dolichotrichum*, *C. microcephalum*, and *C. pachypodioides* Greathouse and Ames (4); *C. tetrasporum* Hughes (5); *C. turgidopilosum*, *C. cristatum*, *C. gangligerum*, *C. velutinum* (syn. *C. brasiliense* (12, p. 787)), *C. atrobrunneum*, *C. seminudum*, *C. cupreum* (referred to *C. trilaterale* (12, p. 807)), *C. causiaeformis*, and *C. succineum* Ames (1); and *C. thermophile* La Touche (6).

The two *Chaetomium* species isolated from Norwegian soils could not be referred to any of the known *Chaetomium* species, and they are therefore proposed as new with the following diagnoses:

Chaetomium homopilatum sp. nov. FIG. 1a–c. FIG. 2a, b.

Perithecia atro-brunnea, ovali, 242–345 × 127–196 μ; rhizoidea numerosa, brunnea; pili laterales et pili terminales nihil divergentes, recti vel minuter undulati, septati, ad basin circ. 4 μ, flaveo-brunnei, ad basin fusci rubescentes, ad apicem hyalini, lenes vel asprelli, nonnulli pili praesertim laterales fortiter sed partim asperi, praecipue paulo supra basin, proiectis globosis; asci obtuso-clavati, octospori; ascosporae fumide coloratae, late ovales, utrinque acutae, 5.9–6.8 × 4.8–6.2 μ. In mycelio intramatricali aleuriosporae, flavobrunneae, globosae, 6.3–8.4 μ diam., ovales 8.4 × 6.3 μ, chlamydosporae intercalares 10.5–15.8 × 4.6–8.4 μ.

Hab.: Isolatum ex terra humosa. Norvegia Occ.

Colonies hyaline, becoming brown where the chlamydospores are formed; aerial mycelium sparse, white. Perithecia black-brown, oval, 242–345 × 127–196 μ with numerous brown rhizoids. Lateral and terminal hairs not different, straight to somewhat undulate, septate, at the base ca. 4 μ, yellow-brown, dark at base with a reddish tint, tip hyaline, smooth to somewhat roughened, some hairs, especially lateral, strongly but partly roughened, with round projections especially at some distance from the base. Asci blunt club-shaped, 8-spored. Ascospores smoke-colored with violet tint, broadly oval, apiculate at both ends, 5.9–6.8 × 4.8–6.2 μ. Some giant spores between the others, about 11 × 6.6 μ. Aleuriospores in the agar-substrate, yellow-brown, globose, 6.3–8.4 μ in diam.; oval 8.4 × 6.3 μ; intercalary chlamydospores 10.5–15.8 × 4.6–8.4 μ.

Hab.: Isolated on filter paper from soil, Western Norway.

Chaetomium homopilatum resembles *C. seminudum* Ames in having uniform lateral and terminal straight hairs, and in producing chlamydospores. According to Ames (1) "myriads of chlamydospore-like bodies 10–15 μ in diameter are produced" in *C. seminudum*. In the form of the perithecia the two species also to some degree resemble each other. *C. homopilatum* differs from *C. seminudum* in having far larger perithecia, more and dark-colored, partly roughened hairs, brown rhizoids and smaller ascospores.

Type culture sent to the Centraalbureau voor Schimmelcultures, Baarn.

The isolate described here as *Chaetomium homopilatum*, in the previous paper was named *Chaetomium sp. 1*.
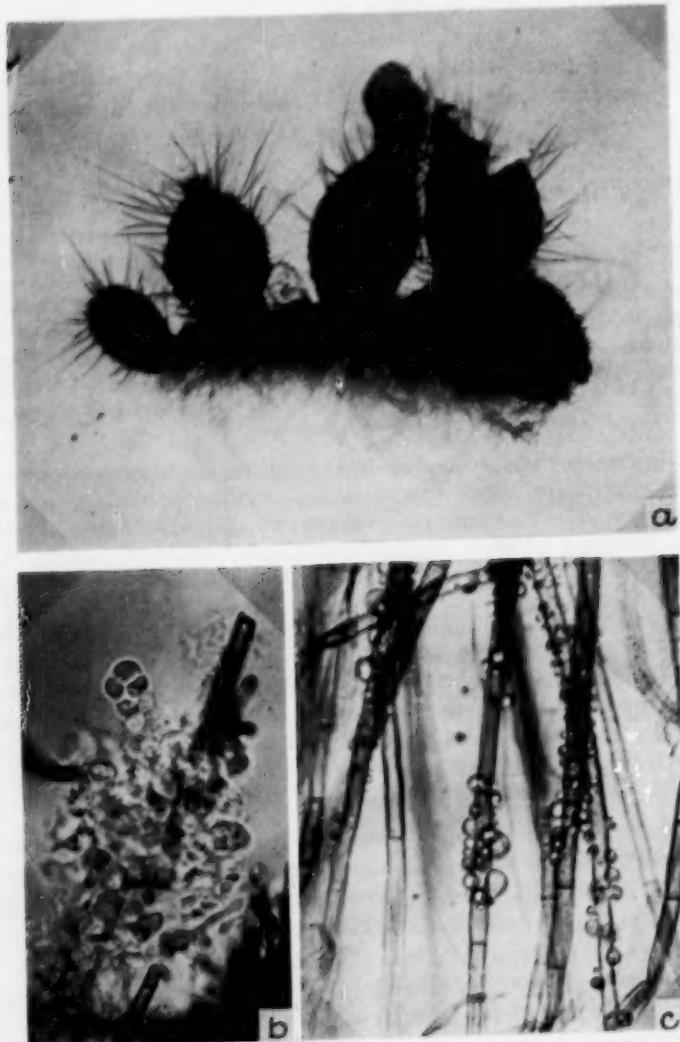

FIG. 1. *Chaetomium homopilatum.* *a.* Perithecia, × 77. *b.* Asci, × 700. *c.* Perithecial hairs with projections, × 700.

Chaetomium flavum sp. nov. Fig. 3a–c. Fig. 4.

Colonia initio flava postea pallescens vel flavo-alba; perithecia nigro-brunnea, globosa vel subglobosa, 276–345 × 230–311 μ; nulla rhizoidea; pili laterales breviores et pauciores quam pili terminales; pili laterales et pili terminales ceterum nihil divergentes, septati, violaceo-brunnei vel flavo-brunnei, trium specierum: a) undulato-spirales, fusci, asprelli, apice obtuso, basi circ. 2.9 μ; b) inferne undulato-spirales, basi fusci, asprelli, parte superiore flavo-brunnei, apicem longum, tenuem, hyalinum formantes, basi circ. 4 μ; c) recti, ad basin fusci, aspelli, parte superiore flavo-
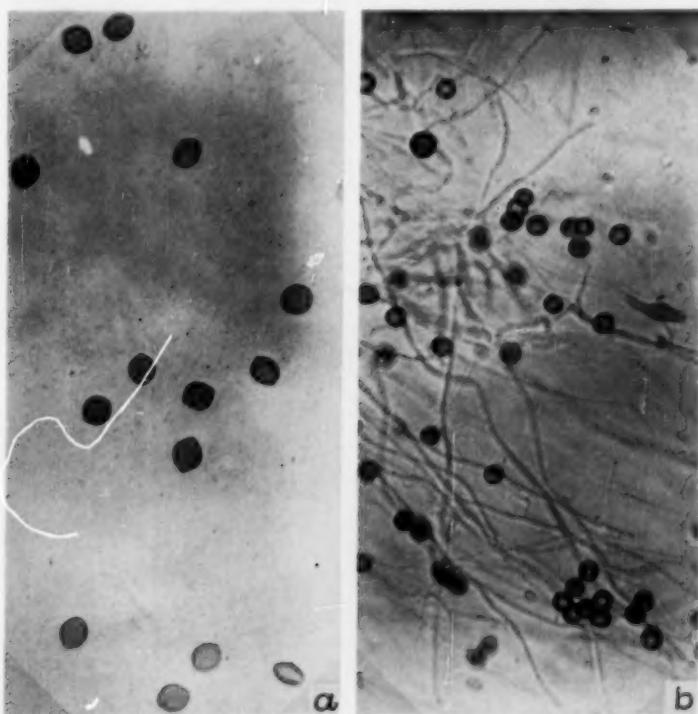
Fig. 2.   *Chaetomium homopilatum.*   a. Ascospores, × 700.
b. Spores in the agar-substrate. × 357.

brunnei, apicem longum, tenuem, hyalinum formantes, basi circ. 4 μ; pili laterales longiores et frequentiores ad apicem perithecii, paullatim commiscentes cum pilis terminalibus; pili terminales numerosi massam dense intertexta efficientes, ex qua pili recti et apices pilorum undulato-spiralium partim superantes; asci clavati; ascosporae fusco-griseo-violaceae; inequaliter limoniformes, utrinque fortiter um-bonatae, 11.9–15.4 × 7.7–8.8 μ.

Hab.: Isolatum ex terra humosa.   Norvegia Occ.
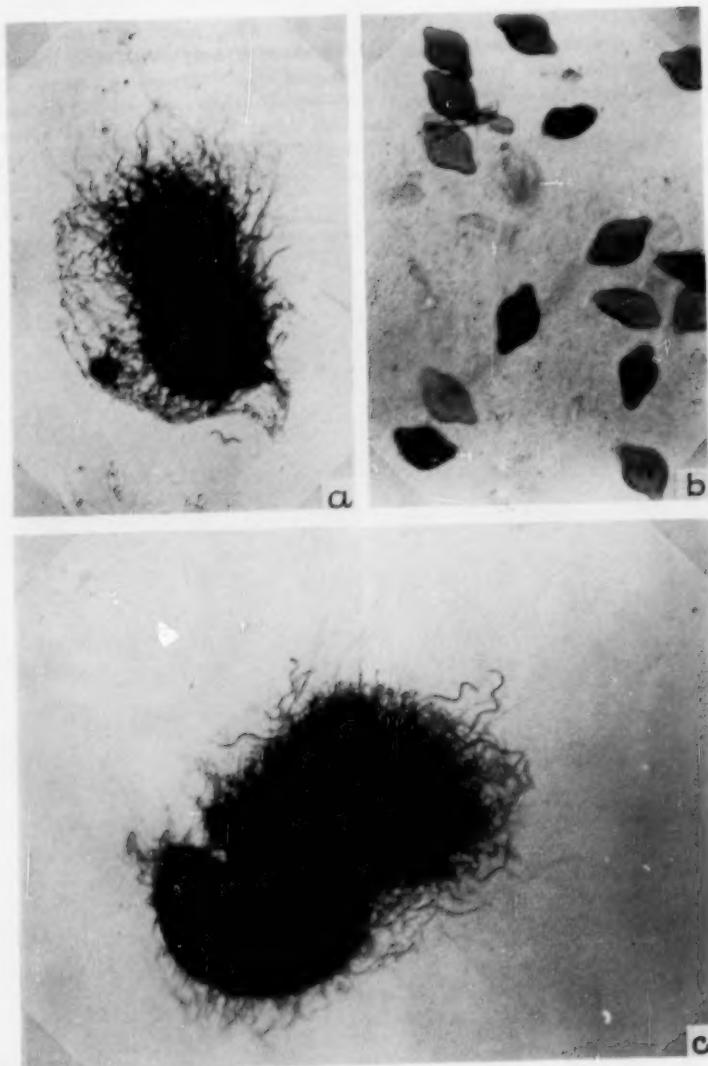
FIG. 3. *Chaetomium flavum.* *a.* Nearly mature perithecium, ×72.
b. Ascospores, ×700. *c.* Mature perithecium, ×77.

Colonies at first yellow, later bleaching to a yellow-white. Perithecia black-brown, globose to subglobose, $276–345 \times 230–311 \mu$. Rhizoids none. Lateral hairs shorter and fewer than terminal; lateral and terminal hairs otherwise not different, septate, violet-brown to yellow-brown, 3 types: a) spirally coiled, dark, somewhat roughened, with blunt tip, at base ca. 2.9μ; b) spirally coiled below, dark at base, roughened, yellow-brown above, tapering to a long, thin, hyaline tip, at base ca. 4μ; c) straight, dark at base, roughened, yellow-brown above, tapering to a long, thin, hyaline tip, at base ca. 4μ. Lateral hairs becoming longer and more numerous towards the end of the perithecia, intermingling with the ter-
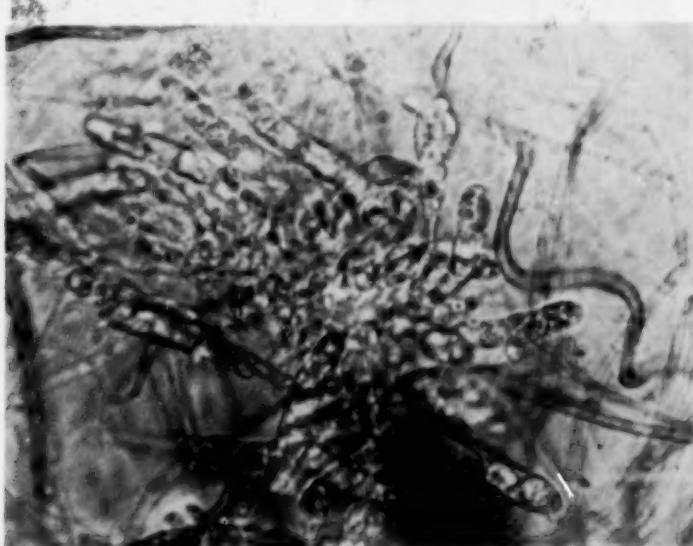
Fig. 4. *Chaetomium flavum.* Immature asci, $\times 700$.

minal hairs. Terminal hairs forming a dense interwoven mass, from which the straight hairs and the straight part of the partly spirally coiled hairs emerge. Asci club-shaped. Ascospores dark grey-violet, irregularly lemon-shaped, strongly umbonate at both ends, $11.9–15.4 \times 7.7–8.8 \mu$. Some giant spores between the others, about $17.6 \times 11 \mu$.

Hab.: Isolated on filter paper from soil, Western Norway.

Chaetomium flavum resembles *C. ochraceum* but differs from it in having smaller perithecia, broader and septate hairs, and lemon-shaped, strongly umbonate and larger spores.

Type culture sent to the Centraalbureau voor Schimmelcultures, Baarn.
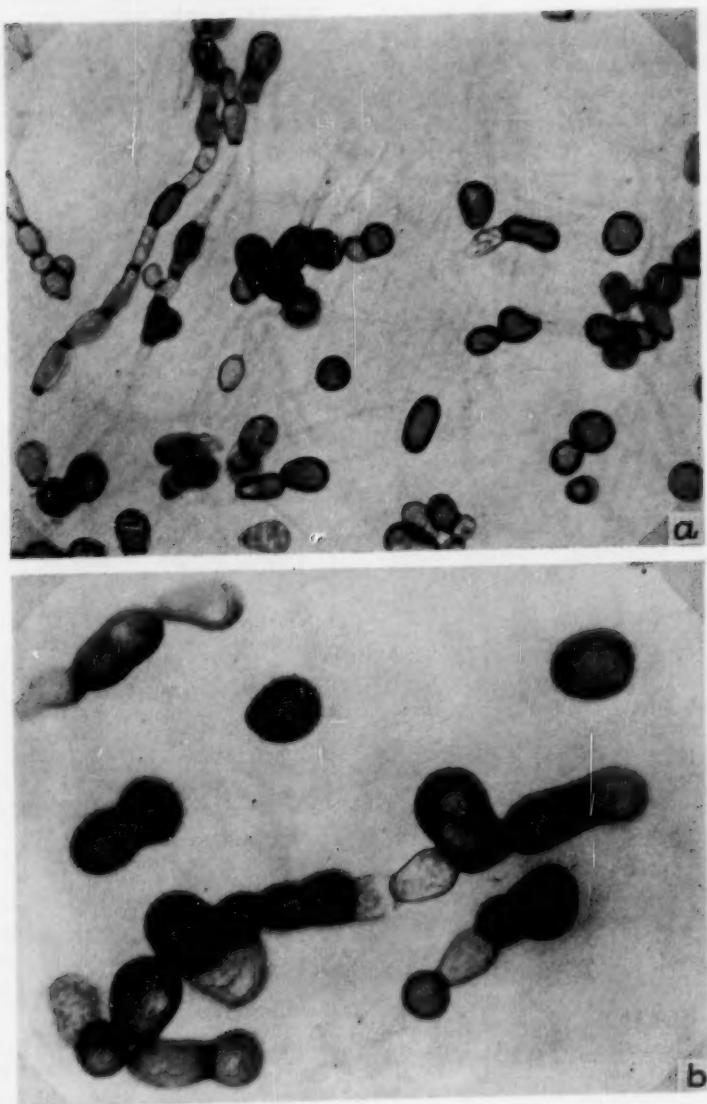
FIG. 5. *Humicola nigrescens.* Spores. *a.* × 357. *b.* × 700.

The isolate here described as *Chaetomium flavum*, in the previous paper was named *Chaetomium sp. II*.

The genus *Humicola* was erected by Traaen (**14**) in 1914. Two species of Humicola were described, *H. fuscoatra* and *H. grisea*.

In 1941, Mason (**8**) proposed *Humicola* Traaen as a synonym of *Monotospora* Corda and *Humicola grisea* as a synonym of *Montospora daleae* Mason, this species name for the first time appearing in 1933 (**7**). A discussion on the taxonomy of *Humicola* and *Monotospora* followed. Here reference is given only to a review on the subject in a recent paper of White and Downing (**17**). The two authors, crediting Mason "with the sorting out of the cultures involved in the early history of *Humicola grisea*," conclude by accepting *Humicola*, with *H. fuscoatra* as the generic type. In the author's opinion, also, this acceptance is justified.

The *Humicola* species isolated from Norwegian soil by the author could not be referred to any of the known *Humicola* species, and is proposed as new with the following diagnosis:

Humicola nigrescens sp. nov.   Fig. 5*a, b*.

> Colonia celeriter increscens; sensim griseo-nigrescens chlamydosporis numerosis et in mycelio ascendenti increscentibus; aleuriosporae fusco-olivaceo-brunneae, singulae, binae, sive catenulatae, globosae 11.9–19.8 μ diam, ovales 12.1–24.2 × 10.6–17.6 μ; chlamydosporae intercalares frequentes, singulae, sive catenulatae, forma et magnitudine multum variantes, saepe cylindriformes.

> Hab: Isolatum ex terra humosa.   Norvegia Occ.

Colonies quickly growing; gradually grey-black due to numerous chlamydospores also being formed in the aerial mycelium.   Aleuriospores dark olive-brown, single, in pairs or in a row, globose 11.9–19.8 μ in diam., oval 12.1–24.2 × 10.6–17.6 μ.   Intercalary chlamydospores frequent, single or in a row, greatly variable in form and size, often cylindrical. Conidia have not been discerned.

Hab.: Isolated on filter paper from soil, Western Norway.

Humicola nigrescens resembles *H. grisea*, but differs from it in having larger spores, and in not forming conidia, which in *H. grisea* are produced in abundance.   Further, there is a distinct difference in the color of the colonies of the two species, *H. grisea* forming a light gray cover of aerial mycelium with a white, dust-like powder due to the conidia, while *H. nigrescens*, at first producing white aerial mycelium, gradually becomes dark black-gray.   The numerous chains of intercalary chlamydospores also distinguish *H. nigrescens* from *H. grisea*.

The isolate has been examined by Dr. E. W. Mason and by professor Traaen, and both agree with the author's opinion that it is a *Humicola* species previously not described.

Type culture sent to the Centraalbureau voor Schimmelcultures, Baarn.

The isolate, described here as *Humicola nigrescens,* in the previous paper was named *Humicola sp.*

All characters of the three new species are based on petri-dish cultures grown on beer-wort agar. The photomicrographs are made from slides mounted in 10% NaOH.

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NOTES ON MYRIANGIACEOUS FUNGI. I

M. J. Thirumalachar and M. J. Narasimhan

(with 5 figures)

1. A new dothioraceous parasite on Phyllachora

The perithecial stroma of *Phyllachora amphidyma* Penz. & Sacc. on *Salacia* sp. collected in Coorg, South India, was found to be parasitized by a member of the Dothioraceae. The stromata of the *Phyllachora* were distributed on the leaves and the infected stromata bore the fruiting of the hyperparasite. The fructification was discoid, cream yellow in color, soft in consistency, and 2 to 3 mm in diameter. The margin of the discoid structure was slightly raised, which resulted in the formation of a cup-like structure in the center (Fig. 1). When several of the stromata closely situated were parasitized, there was a cluster of cupulate structures formed. In the early stages of development, the young fruit body was seated on a weft of hyphae.

Sections through the infected *Phyllachora* stroma revealed the complete suppression of asci formation. The mycelium of the hyperparasite completely filled the perithecial cavity and spread on the sides. Bundles of hyphae connected the fruit body seated above with the perithecia (Fig. 2).

The asci are formed in a parallel layer in separate locules. They are clavate to fusoid, separated by elongated interthecial paraphysoids (Fig. 2). The ascospores are fusiform, tapering on both ends, hyaline, biseriate and 3-septate. The apices of the ascospores are pointed or elongated into filamentous structure (Figs. 4, 5).

The parasitic habit of the fungus on *Phyllachora* and the type of fructification containing a single layer of asci, separated by interthecial paraphysoids, indicate close relationship with *Dothiora subtropica* (Wint.) Miller & Burton (**1943**). It differs from *Dothiora*, however, in the type of stroma (which is dark and brittle in *Dothiora*) and the ascospore septation. The striking resemblance of *Dothiora* to discomycetous fungi has been pointed out by Miller and Burton, who discuss the position of the genus assigned by different investigators. They place it as an advanced form of the Myriangiales, where asci are no longer globose and are grouped in a single layer. But in both *Dothiora*
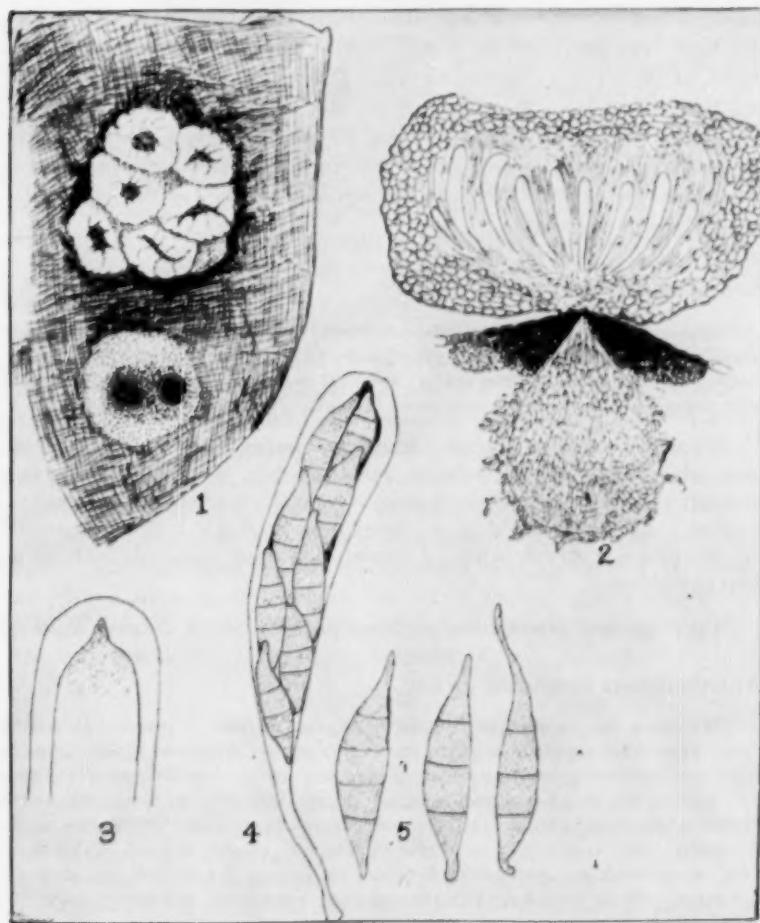
FIGS. 1–5. *Annajenkinsia fungicola.* 1. Enlarged view of ascomata on the stroma of *Phyllachora amphidyma*, × 15. 2. Median section through the ascomata showing attachment to the stroma of *Phyllachora*, × 75. 3. Apex of ascus, × 1150. 4. Ascus showing distichus arrangement of ascospores, × 750. 5. Ascospores, × 1150.

and the fungus under study, the asci have a single wall layer as against the typical double walls of myriangiaceous asci.

The fungus under study is a member of the Dothioraceae of Theissen & Sydow (**1917**) and comes close to *Lepidothiora* v. Höhn. which is

distinguished from *Dothiora* by its hyaline phragmospores. Two species have been described by von Höhnel. The stromata, however, are stated to be (cf. *L. elliptica* (Fuck.) v. Höhn.) dark as in *Dothiora* and this differs from the condition present in the fungus under study. The dothioraceous fungus parasitizing *Phyllachora amphidyma* is therefore accommodated in a new genus with the name *Annajenkinsia*, named in honor of Dr. Anna E. Jenkins, distinguished student of the myriangiaceous fungi of the world.

Annajenkinsia gen. nov.

Mycelium parasiticum, innatum; ascomata erumpentia, superficialia, pallide lutea, gelatinoso-carnosa, discoidea vel cupulata, ad marginem elevata, glabra; hypothecia parenchymatica, tenuiter crassa; epithecia hyalina. Asci unistratose oriundi inter paraphysoidea, 8-spora; ascosporae biseriatae, hyalinae, multiseptatae.

Mycelium parasitic, innate. Acsoma erumpent, superficial, pale yellow, soft, discoid, cupulate due to raised margin, smooth; hypothecium present, thin-walled, hyaline, parenchymatous; epithecium thin-walled, hyaline. Asci in a single layer, developing in single locules, separated by interthecial paraphysoids, 8-spored, biseriate; ascospores hyaline, phragmospores.

Type species: *Annajenkinsia fungicola* Thirum. & Narasimhan.

Annajenkinsia fungicola sp. nov.

Mycelium in stromatibus *Phyllachorae amphidymae* cujusdam parasitans, dense gregarium, matricis stromata omnino obtegens. Ascomata superficialia, ex hyphis radiantibus oriunda, contextu gelatinoso-carnoso, cupuliformia vel discum pallidissime luteum, efformantia, margine elevata, 500–600 μ in diam., 600 μ alta. Hypothecium hyalinum, minutissime pseudoparenchymaticum; epithecium tenue, hyalinum. Asci unistratosi, in loculis singularibus oriundi, 8-spori, 92–130 × 13–18 μ, crasse tunicati. Ascosporae distichae, fusiformae, 3–4-septatae, ad septa vix constrictae, 25–34 × 6.5–8 μ. Paraphysoides sat numerosae, fibroso-cellulosae.

Mycelium parasitic, innate, completely replacing the asci of *Phyllachora amphidyma*. Ascomata superficial, seated on an emerging weft of hyphae, pale-yellow, soft, discoid to cupulate, raised along the margin, 500–600 μ broad, 600 μ high, hypothecium parenchymatous; epithecium thin-walled and hyaline; asci in a single layer developing in single locules, separated by interthecial paraphysoids, 92–130 × 13–18 μ, 8-spored, biseriate. Ascospores hyaline, fusiform, thin-walled, 3–4-septate, not constricted at the septa, 25–34 × 6.5–8 μ.

Hab. on *Phyllachora amphidyma* on *Salacia* sp., Coorg, South India, 10th March, 1948 (Type).

2. On Masonia Hansford and Angiotheca Syd.

The genus *Angiotheca* was proposed by Sydow (**1939**) with *A. scabra* Syd. as type, for an epiphyllous Myriangiaceous fungus on *Antiaris africana* (Urticaceae) collected by Dr. F. C. Deighton from the Gold Coast, Africa. The fungus was parasitic on leaves with exhyphopodite, dematoid, superficial mycelium, bearing non-ostiolate, perithecium-like structures. These possessed brownish-black outer parenchyma and inner fibrous layer, with a single layer of asci developing as in Myriangiales. The spores were described as muriform and dark-brown.

Masonia Hansf. was described by Hansford (**1944**) from Uganda, Africa, on *Chlorophora excelsa* (Urticaceae) with the following characters: The fungus is parasitic with external, olivaceous exhyphopodite mycelium, bearing superficial, non-ostiolate globose ascomata, dark-brown externally and bearing interiorly a single monascigenous layer as in the Myriangiales, and possessing dark-brown ascospores. Since the generic descriptions read very similarly, the type specimens were compared by analyzing the descriptions given by Hansford and by Sydow. The type specimens of both *Angiotheca scabra* and *Masonia chlorophorae* were made available for study through the kindness of Mr. E. W. Mason while the senior author had the opportunity to study at the Commonwealth Mycological Institute, Kew, England.

	Angiotheca Syd.	*Masonia* Hansf.
	Type: *A. scabra* Syd.	*M. chlorophorae* Hansf.
1. Type locality:	W. Africa	E. Africa
2. Host family:	Urticaceae	Urticaceae
3. Mycelium:	dermatoid, exhyphopodite	olivaceous, exhyphopodite
4. Fruit body:	nonostiolate	nonostiolate
color:	dark-brown	dark brown
size:	150–280 × 100–190 μ	80–150 μ diam. × 50 μ high
surface:	perithecia warty	perithecia setose
asci:	single layer and uniloculate	single layer and uniloculate
size:	60–85 × 35–50 μ	40–45 μ
ascospores:	muriform, 6–9 transverse septa and 2–3 vertical ones, dark-brown, 30–38.5 × 14–16 μ	muriform, 6–8 transverse and 1–2 vertical ones, dark-brown, 30–34 × 13–16 μ

From the above analysis it appears possible that the two fungi are congeneric or even identical with each other. Examination of the type specimens of the two genera has indicated that both of them are super-

ficial ectoparasites with the perisporiaceous type of hyphae, the same
type of stromata, asci, etc. and parasitizing two closely related members
of the Urticaceae in Africa. In *Angiotheca* the hairs on the perithecia
are straight while they are slightly curved in *Masonia* in a few cases.
This difference is only of specific significance and therefore *Masonia*
Hansf. may be treated as being congeneric with the earlier described
Angiotheca Syd.

In conclusion the senior author wishes to acknowledge his gratitude
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NEW AND INTERESTING SPECIES OF BASIDIOMYCETES. IV[1]

R. SINGER

(WITH 8 FIGURES)

Favolaschia dealbata sp. nov.

Pileo aqua reimbuto melleo vel armeniaco-alutaceo, exsiccato albo, levi vel leniter tesselato, 2–2.5 mm lato. Poris adultis non rotundatis, 0.1–0.5 mm diam., plus minusve 30. Pseudostipite puncto excentrico superficiali pilei affixo, 1–1.5 × 0.5 mm. Sporis 7.5–9(–11.5) × 5.7–7.2(–8.5) μ, amyloideis, ellipsoideis vel subglobosis, levibus; basidiis 25 × 7.8 μ, tetrasporis; gloeocystidiis in hymenio nec non ad superfies steriles numerosis, clavatis, in vascula gleophora non prolongatis, aureolo-melleis (KOH), gradatim pallescentibus, 25–32 × 8.5–13.5 μ; vasculis gloeophoris admodum sparsis in pseudostipite tramateque hymenophorali, tenuibus; dendrophysibus in superficiebus sterilibus et ad poros numerosis, hyalinis vel (rarissime) pallide melleis, 18–31 × 14–19.2 μ, in superficiebus pororum apiculis ± 1 μ longis ornatis, ceterum 1–2.5(–3.5) μ longis, intus non amyloideis nec caeruleo cresylico atrocaerulescentibus, late cylindraceis vel saepius ellipsoideo-vesiculosis, pedicellatis necne; pseudophysibus nullis visis; transitionibus inter gloeocystidia et dendrophyses absentibus; tramate pilei hyphis hyalinis, tenuibus, fibulatis, inamyloideis consistente, gelatinoso; tramate pseudostipitis vix gelatinoso. Ad *Bambusam*, Casita Alta supra Boquete, Prov. Chiriqui, Panamae, 31–VII–1952, Martin & Welden *8070* (F). A *F. pygmaea* pseudostipite, dendrophysibus angustioribus, habitatione differt; apiculi dendrophysum frequenter quoque longiores sunt.

Campanella dendrophora sp. nov. FIG. 1.

Pileo caesio vel primum albo, dein caesio, reticulatim ruguloso transparenterque reticulato, convexo, circulari vel conchato, postice plerumque insinuato et ibidem pseudostipite vel stipite affixo concolori, brevi, laterali, 2 × 1 mm; carpophoro toto 10–16 mm lato. Hymenophoro sublamelloideo, anastomosante, usque ad 1.5 mm projiciente, cristis lamelliformibus distantibus. Carne exigua, membranaceo-gelatinosa. Sporis 8.8–10.2 × 6.8–8.2 μ, hyalinis, levibus, guttulis oleaceis exiguis impletis, plerumque triangularibus, rarius elevatione dorsali suprahilari vel excrescentia ventrali praeditis, inamyloideis; basidiis 38–40 × 8.2–10.2 μ, clavatis, sterigmatibus quatuor longis apiculatis; pseudophysibus dendroideis, structuram hypharum epicuticularium Marasmielli in mentem revocantibus, hyphosis, ramosis, coralloide diverticulatis, nonnumquam excrescentiis cystidioideis praeditis, his ultimis capitatis et individualisatis ita ut cystidia denominari debeant; cystidiis illis ventricosis vel cylindraceis, apice semper capitatis, capitulo 5.5–9 μ diametro, ceterum 40–53 ×

5.5–11 μ, parte collari plus minusve 4 μ crassa, parietibus quam in pseudophysibus subcrassioribus intraque quam in pseudophysibus solutione phloxina obscurius colorabilibus, parte basali saepe diverticulata in cystidiis minus individualisatis cylindrico-capitatis; metuloideis crasse tunicatis *Campanellae simulantis* modo etiam praesentibus, opacis, hyalinis, cylindraceis vel frequentius fusoideo-ventricosis vel fusoideo-ampullaceis, apice rotundatis, moderate numerosis, apice crystalline incrustatis, 35–44 × 8.5–9.7 μ; hyphis tramatis tenui-tunicatis, filamentosis, in massa gelatinosa laxe dispositis, hyalinis, fibulatis, haud amyloideis; epicute pilei structura asterostromelloidea gaudente, ex hyphis simplicibus vel ramosis vel irregularibus ex toto diverticulatis composita. Ad culmos *Chusqueae couleu*, Argentina, Neuquén, Puerto Manzano, Maio 18, 1952, Singer *M746* (LIL), TYPUS. Isla Victoria, Maio 17, 1952, Singer *M706* (LIL), PARATYPUS.
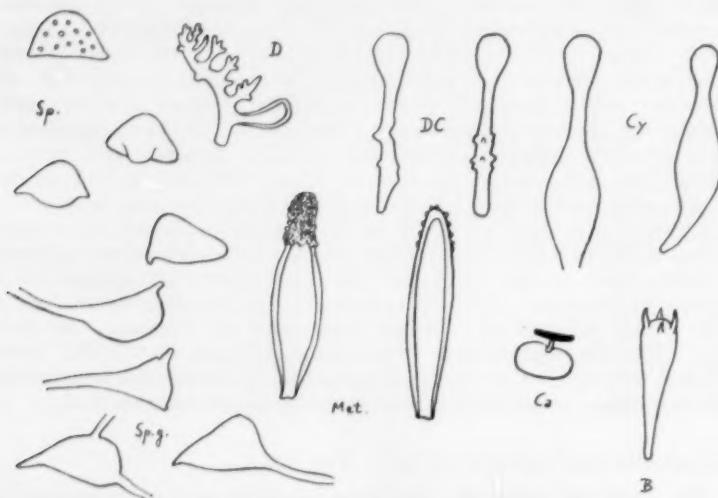

FIG. 1. *Campanella dendrophora*. *Sp.* Mature spores, × 1100. *Sp.g.* Same, germinating. *D.* Dendrophysoid elements, × 750. *DC.* Dendrophysoid cystidia, × 750. *Cy.* Cystidia, × 750. *Met.* Metuloids, × 750. *Ca.* Carpophore, × ½. *B.* Basidium, × 500.

Campanella tenuitunicata sp. nov.

Pileo albo, postice fuscidulo, profunde canaliculato-sulcato reticulatoque, glabro, conchato, 5–17 mm lato. Hymenophoro lamelloideo, lamellulis minus projicientibus anastomosisque connexo, elevationibus lamelloideis distantibus, sordide albo. Stipite nullo. Odore nullo. Sporis 6.8–8.3 × 3.5–4.8 μ, hyalinis, admodum irregularibus forma, aut ellipsoideis vel apice mucronatis e fronte visis, lateraliter visis elevatione suprahilari externa (dorsali) calcariformi plerumque praeditis, guttula olei saepe in parte inferiore latiore sporarum excentrice collocata, latere interiore (ventrali) convexo vel applanato vel medio depresso, membrana inamyloidea, levi; basidiis

27–34 × 6.8–8.5 μ, clavatis, hyalinis, tetrasporis; basidiolis cylindraceis vel anguste clavatis, raro subfusoideis; cystidiis hyalinis, tenuitunicatis, in tertia inferiore incrassatis, centro constrictis, apice plerumque capitatis vel subcapitatis levibus vel incrustatione debili instructis, 30–33 × 6-7 μ, in parte constricta usque ad 2–3.5μ attenuatis, apice 4.8–6.2 μ latis; hyphis tramatis gelatinascentibus, hyalinis, tenuibus, tenuitunicatis, vel parietibus subtenuiusculis instructis, inamyloideis, fibulatis; epicute pilei structura asterostromelloidea gaudente, pallidissime mellea. Ad *Schinum* sp., in regione montano-subxerophytica sed aestate pluviosa. Argentina, prov. Catamarca, Suncho, Santa Rosa, vallis Rio Campo, 1650 m. alt., Jan. 18, 1952, Singer *T1750* (LIL), TYPUS.

CAMPANELLA CANDIDA (A. L. Smith) Sing. var. **stipitata** var. nov.

Pileo candido, canaliculato-reticulato, glabro, subcirculari, membranaceo tenuique, convexo vel irregulari, 10 mm lato. Hymenophoro lamelloso, sordide albo, leniter subbrunnescente in siccis, lamellis distantibus, adnexis vel adnatis, angustis,

FIG. 2. *Ca.* Carpophore of *Collybia paranaguae*, schem. × 1. *Ch.* Cheilocystidium of *Collybia paranaguae*, × 2000. *Ca.s.* Dried carpophores of *Clitocybe kabulensis*, × 1.

lamellulis angustioribus cum lamellis anastomosantibus, anastomosis venosis. Stipite grisello-albo, tomentoso, horizontali, fortiter excentrico vel laterali, 2 × 0.7 mm. Carne alba, gelatinosa, tenui, membranacea, inodora. Sporis (7–)9 × (4.5–) 4.8(–6.2) μ, ellipsoideis, hyalinis, inamyloideis, levibus; basidiis 35–38.5 × 6.8-8.7 μ, clavatis, tetrasporis, paucis bisporis; cystidiis ditopis, (1) eis *Campanellae tenuitunicatae* analogis, sed frequenter crasse tunicatis, hyalinis, centro constrictis (constrictione e. gr. 2.8 μ crassa), plus minusve 64 μ longis, 7–7.5 μ latis, (2) eis *Campanellae simulantis* analogis, metuloideis, crasse tunicatis (3.5 μ), raro subtenuitunicatis, saepe subsolidis, opacis, fusoideo-ventricoso-ampullaceis, pedicellatis necne, acutis vel obtusis, incrustatione crystallina apicali mellea instructis, 37–55 × 9.5-13.7 μ; hyphis tramalibus in massa gelatinosa fluentibus; epicute asterostromelloidea; fibulis praesentibus. Ad ramulos (*Schini?*). Santa Rosa, Vallis Rio Campo, Catamarca, Argentina, 1650 m. alt., Jan. 18, 1954 (LIL) TYPUS.

Clitocybe augustinensis sp. nov. FIG. 3.

Pileo albo, dein Clitocybarum modo pallide aurantio-alutaceo siccante, glabro, levi, infundibuliformi, margine incurvo, 24–50 mm lato; epicute ex hyphis tenuibus, tenuitunicatis repentibus formata; lamellis albis in siccis lutescentibus, confertissimis, angustis, intermixtis, profunde decurrentibus; sporis 4.7–5.2(–7) × (2–)2.2–3 μ, levibus, ellipsoideis vel ellipsoideo-oblongis, hyalinis, levibus, inamyloides; basidiis 20 × 3.8–4 μ, tetrasporis; cystidiis nullis; tramate hymenophorali structura Clitocybarum typi gaudente; subhymenio vix evoluto; stipite albo, gracili, duodecim e basi bulbosa squamosa alba ecrescentibus; carne albida ex hyphis fibulatis inamy-
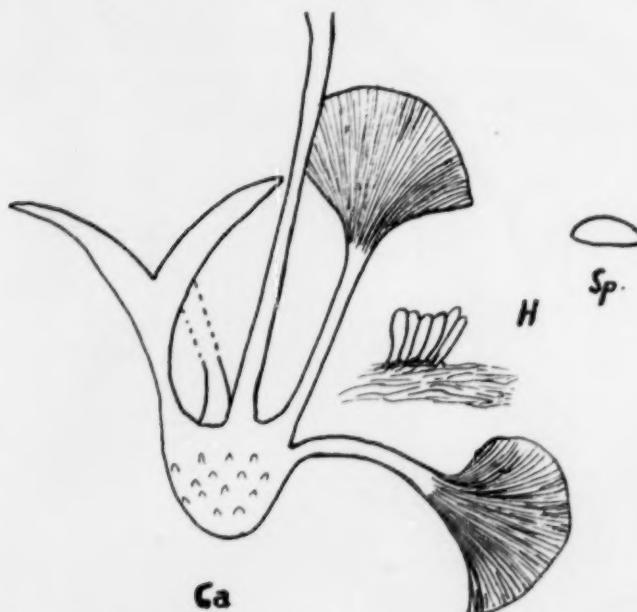

FIG. 3. *Clitocybe augustinensis*. *Ca*. Carpophore, × 1. *Sp*. Spores, × 2000. *H*. Fragment of hymenium with hymenophoral trama, × 500.

loideis tenui-tunicatis, haud gelatinosis formata. In fimosis ruderatis, St. Augustine, Trinidad, J. Wilson 9–II–1950, Dennis comm. (sub *Lentodiello concavo*), no. *481.* (K).

Clitocybe kabulensis sp. nov. FIG. 2, *Ca.s.*

Pileo flavido-albo, sicco, sordide albo, levi, glabro, opaco, profunde infundibuliformi, excentrico. Lamellis flavido-albis, siccis albidis, satis angustis, confertis, profunde decurrentibus. Stipite flavido-albido, levi, glabro, breviusculo. Carne albida. Sporis variabillimis, plerumque ovoideis vel ellipsoideis, 7.5–9.5(–10) × 4–5.7(–6.4) μ, ceteris cylindraceis, 7.5–12(–14.5) × 4.3–4.7 μ, omnibus hyalinis,

levibus, inamyloideis; basidiis 30 × 8 μ, maturis basidiolis multo majoribus, clavatis, hyalinis, tetrasporis; cystidiis cheilocystidiisque nullis; subhymenio ex elementis filamentosis admodum irregularibus, tenuibus, hyalinis consistente; tramate hymenophorali regulari vel subregulari ex hyphis haud divergentibus, elongatis, subintertexto-subparallelis undulatisque, haud gelatinosis, vix crasso-tunicatis (membrana 0.4 μ crassa) consistente, hinc haud typice clitocybeoideo; epicute pilei ex hyphis appressis elongatis, hyalinis, levibus (interdum minutissime asperulatis), haud diverticulatis nec nodosis consistente; hyphis omnibus inamyloideis, fibulatis. Ad *Labiatas* in collina arenosa. Afganistan, Tangi gharu, prope Kabul, 1770 m. alt., Maio 12, 1950, Gilli no. LXXXVI (W), TYPUS.

Clitocybe subulifera sp. nov.

Pileo atrobrunneo ("Mohawk" M & P) in centro, marginem versus multum pallidiore, haud viscido, vix hygrophano vel subhygrophano, glabro, nudo, margine breviter et haud manifeste striatulo transparentia, ceterum levissimo, convexo dein convexo-applanato, subumbonato, 36–50 mm lato. Lamellis hyalino-albidis, fortiter intermixtis, saepe nonnullis furcatis intermixtis, moderate latis vel satis angustis (2.5–4.5 mm), linearibus, stipiti variabiliter affixis, abrupte sed haud profundo emarginatis vel adnato-subdecurrentibus (manifestius decurrentibus in siccis), acie subcrenulatis vel rarius integris; sporis in massa candidis. Stipite albo, lenissime fibrilloso-subpruinato ad apicem, ceterum glabro nudoque, solido, demum subcavo, haud tubuloso, molli, haud cartilagineo nec fragili, subaequali vel apicem versus subattenuato, 30–40 × 6–9 mm. Carne alba, immutabili; sapore miti; odore nullo. Sporis 4–4.8 × 3–3.5 μ, uninucleatis, guttula oleacea una plerumque praesente, hyalinis, inamyloideis, levibus, subglobosis vel breviter ellipsoideis; basidiis 25–32 × 6.8–7.5 μ, granulis carminophilis destitutis, clavatis, tetrasporis; cystidiis ad latera aciesque lamellarum praesentibus, subulatis, solutione phloxina facile coloratis, contentu protoplasmatico optice destitutis, levibus, 10–15 μ projicientibus, ex parte infima subhymenii natis, 33–41 × 7.5–9 μ; acie haud heteromorpha; tramate hymenophorali in parte interiore majore ex hyphis parallelis vel subparallelis, saepe leniter ventricosis (sed haud vesiculosis) latiusculisque consistente, in parte subhymnio proxima ex hyphis angustis paulum versus hymenium divergentibus; subhymenio angusto, ex elementis elongatis sed exiguis, ramoso-intertextis consistente; epicute angusta, ex hyphis intertextis tenuibus (2–3 μ diam.) hyalinis, densiusculis consistente; hypodermio crassiore quam epicutis pigmento incrustante carente, ex hyphis eis epicutis crassioribus magisque parallelis consistente; hyphis omnibus fibulatis, haud amyloideis. Ad humum silvestrem in silva subxerophytica *Eugeniae, Piptadeniae, Celtidis,* etc., gregatim. Argentina, prov. Tucumán, De "Tapia" vs. mer. Febr. 17, 1951, Singer *T1220* (LIL), TYPUS. Colore *Tricholomatis pessundati* gaudet, habitu *Lepistam glabellam* simulat.

Collybia albelloavellanea sp. nov.

Pileo albo vel albido, centro cinnamomeo vel pallide avellaneo, estriato, levi, glabro, convexo, 3.5–8.5. Lamellis albis, subconfertis vel mediocriter confertis, confertis in vivis juvenilibus, angustis, subliberis vel late adnexis. Stipite dense avellaneo, apicem versus pallidiore, pruinato-subvellereo de apice ad basin, basi aut attenuata aut bulbosa; mycelio albo. Carne alba; odore nullo. Sporis 6–7.7 × 2.5–2.8 μ, levibus, hyalinis, oblongis, intus applanatis; basidiis 19.5–20 × 4.5–5 μ;

cheilocystidiis inconspicuis, filamentosis vel subbasidiomorphis, sparsis; cystidiis nullis; epicute pilei ex hyphis repentibus, filamentosis, levibus, hyalinis consistente; vestimento stipitis pilis erectis crasse tunicatis, subhyalinis vel hyalinis levibus formato; pilis subulatis vel centro subventricosis, semper apicem versus attenuatis, acutis vel saepius obtusis, 38–55×3.2–$5.5\,\mu$; tramate hymenophorali regulari, interdum subintertextis hyphis formato; fibulis numerosis. Ad lignum emortuum in silva tropicali litorali Paranaguá Brasiliae (Paraná), Sept. 27, 1952, Singer *B409* (F), TYPUS.

Collybia paranaguae sp. nov.   FIG. 2, *Ca, Ch.*

Pileo obscure castaneo vel cinnamomeo-fusco, centro pallidiore, hygrophano, subpallido in siccis, glabro, in siccis nitente subsericeo, haud striato in statu humido atque sicco, conico-subapplanato, umbone exiguo praedito, 13 mm lato. Lamellis avellaneis, confertissimis, adnatis (nec non unum ad latus subdecurrentibus), angustis (1 mm latis). Stipite avellaneo vel isabellino-pallido, subglabro, helicis modo minute substriato-sulculato, aequali, 40×1.5 mm; mycelio basali tomentoso haud strigoso, albo. Carne superficie minus colorata, subalbida vel albida, carnoso-tenacella, in stipite tenaci, inodora, miti. Sporis $6 \times 2.7\,\mu$, levibus, hyalinis, inamyloideis; basidiis clavatis, 15–16.3×4.5–$5\,\mu$, tetrasporis; cheilocystidiis paulum conscipcuis, basidiorum magnitudine vel longioribus, subcapitatis vel rarius clavatis, hyalinis, inter basidia sparsis; cystidiis nullis; tramate lamellarum regulari, ex hyphis subparallelis, hyalinis, filamentosis consistente; epicute pilei ex hyphis radiatim dispositis filamentosis, levibus, pigmento intracellulari dissoluto instructis consistente; hyphis omnibus inamyloideis, fibulatis. Ad basin *Palmae* solitario, in silva tropicali litorali. Paranguá Brasiliae (Paraná), Sept. 27, 1952, Singer *B406* (F), TYPUS. *Collybiam trinitatis* Dennis in mentem revocat.

Collybia syringea sp. nov.

Pileo violaceo, dein sordide pallido, levi, glabro, centro demum subalutaceo, campanulato, centro subacuminato-subpapillato, margine magis declivi, 12 mm lato. Lamellis caeruleo-violaceis (ut in multis *Cortinariis*), ventricosis, latis, adnexis, distantibus. Stipite lamellis concolore, superne glabro levique, aequali, farcto, demum cavo, 29×0.7 mm, basin versus 1 mm lato; mycelio basali tomentoso, substrigoso; velo nullo. Carne albida in pileo, lilaceo-violacea in stipite, inodora. Sporis 3.3–4.5×3–$3.5\,\mu$, membrana homogenea inamyloidea instructis, submelleo-hyalinis (NH$_4$OH), tenui-tunicatis, breviter ellipsoideis; basidiis 24–25×5.3–$5.7\,\mu$, clavatis, tetrasporis; cystidiis nullis; cheilocystidiis marginem pilei versus sparsis cylindraceis saepeque subventricosis basin versus (usque ad $6\,\mu$ diam.), 25–30×2.7–$3.5\,\mu$, hyalinis; subhymenio ex hyphis brevibus latiusculis, exiguis, irregulariter dispositis consistente; tramate hymenophorali plus minusve regulari; epicute ex hyphis appressis efformata, in strato hyphis superficialibus supposito plerumque hyphis latis (usque ad $17\,\mu$ diam.) formata, cellulis terminalibus, ubi superficiem adaequant, interdum clavatis, strato superficiali plerumque ex hyphis filamentosis tenuibus efformato, in omnibus membrana submelleo-hyalina vel flavidula notata; hyphis tramatis pilei hyalinis, inamyloideis, fibulatis, binucleatis (ut cheilocystidia); sporis demum binucleatis vel uninucleatis. Ad folia putrida in silva tropicali litorali, Paranaquá, Brazil (est. de Paraná), Sept. 27, 1952, Singer *B404* (F). Species

C. iocephalae probabiliter affinis, *Mycenae syringeae* Murr. nec non *Tricholomati microsporo* sensu Dennis (vix Ell. & Ev.) possibiliter identica.

Pleurocollybia apoda sp. nov.

Pileo pallide ochraceo, in siccis albido vel fulvo- albido, glabro, levi, applanato, 9–42 mm lato. Lamellis albidis, confertissimis, angustis, ad lateralem pilei extensionem concurrentibus; sporis in massa candidis. Stipite nullo. Carne alba. Sporis 2.7–3 × 1.8–2 μ, breviter ellipsoideis, levibus, hyalinis, inamyloideis; basidiis admodum exiguis, e. gr. 9 × 4 μ; crystidiolis (?) ampullaceis 11.5 × 3.5 μ; hyphis tramatis hymenophoralis parallelis, hyalinis; epicute pilei ex hyphis filamentosis cutem efformantibus consistente; hyphis omnibus defibulatis, inamyloideis. Ad lignum putridum, Rio Sardinilla, Zona Canalis, Panamae, Julio 10, 1952, Martin & Weldon *7518* (F, 1A), TYPUS et ISOTYPUS.


Me   Ch
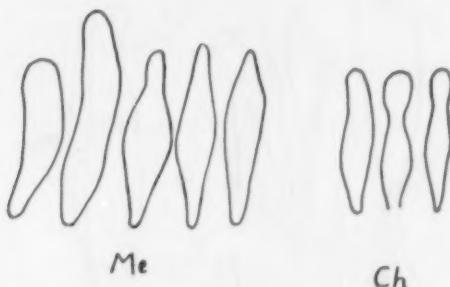
FIG. 4. *Hohenbuehelia nigra* var. *microspora.* *Me.* Metuloids (without incrustation), × 1000. *Ch.* Cheilocystidia, × 1000.

HOHENBUEHLIA NIGRA (Schw.) Sing. var. microspora var. nov. FIG. 4.

Pileo atrogriseo, subtiliter tomentoso (probabiliter ex mycelio basali), resupinate affixo, usque ad 8 mm lato. Lamellis subnigris, acie griseolis, primum subdistantibus, dein confertis vel confertissimis, angustis, concurrentibus attenuatisque loculum excentricum vel lateralem versus, stipitis vestigium nullum. Stipite pseudostipiteque nullo. Sporis in massa candidis, sub microscopio hyalinis, 4–4.5 × 2.5–3.5 μ, ellipsoideis, interdum breviter cylindraceis, haud reniformibus, guttulis repletis, levibus; cheilocystidiis forma metuloides in mentem revocantibus, sed membrana tenuiore instructis, fuscidulo-hyalinis, apice late rotundatis, cylindraceis, saepe medio constrictis, etiam ampullaceis vel clavatis, 18–20 × 3.5–4.5 μ; cystidiis metuloideis fusoideis, ampullaceis, cylindraceo-clavatis, mucronatis, etc., crystallis incrustatis, crasse tunicatis, projicientibus vel vix projicientibus, membrana ipsa mediocriter crassa sed incrustatione crystallina crassa praeditis, quae in KOH cito dissolvitur, numerosis, 23–33 × 5.7–8 μ (sine incrustatione); hyalinis si una cum incrustatione observantur, denudatis fuscidulis; subhymenio ex elementis exiguis inflatis irregularibus consistente; hyphis pilei elongatis, inamyloideis, in massa

gelatinosa undulantibus, epicute pilei haud notabiliter differentiata. Ad ramum emortuum dicotyledoneum vel ad truncum, Corundú, Zona Canalis Panamae, Aug. 13, 1952, Martin & Welden, *8353* (F, 1A) TYPUS et ISOTYPUS.

Hohenbuehelia pycnophylli sp. nov.　Fig. 5.

Pileo atroumbrino, nitente, glabro, levi, forma magnitudineque *H. petaloidem* simulante. Lamellis albido-cremeis in siccis, acie fuligineis, subconfertis, subangustis, intermixtis, decurrentibus.　Stipite ad latus fertile bene evoluto, albido, partim fuscidulo, sed ad latus sterile haud limitato, paulum albido-velutino, magis erecto quam pileus.　Carne alba, in siccis inodora.　Habitu generali *H. petaloidis*. Sporis 7.7–8.3 × 6.3–7.2 μ, hyalinis, levibus, guttulis nonnullis minutis impletis; basidiis (25–)28–29(–43) × 7.5 μ, clavatis, tetrasporis; cheilocystidiis versiformibus,
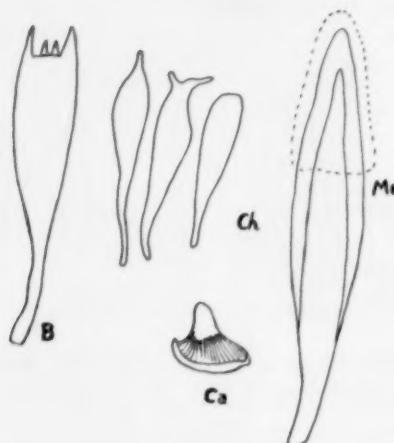
Fig. 5.　*Hohenbuehelia pycnophylli*.　B. Basidium, × 1000.　*Ch*. Cheilocystidia, × 1000.　*Me*. Metuloids, × 1000.　*Ca*. Carpophore, × 1.

plerumque basidiomorphis, uno- vel bi-appendiculatis vel sterigmatoiditer mucronatis vel irregulariter clavatis neque appendiculatis, pallide fuligineis, 22–36 × 4.7–9.3 μ; metuloideis moderate numerosis, tegumento crasso crystalloideo instructis, ut minime in parte superiore, crasse tunicatis (membrana 2–4 μ crassa), fusiformibus, sine incrustatione 40–62 × 8.5–11 μ et hyalinis; hyphis pilei massa gelatinosa circumditis, hyalinis, fibulatis. Ad *Pycnophyllum*. Sec. ascensum ad Abra de Remate via Casa Colorada, prov. Jujuy, dep. Tilcara, Argentinae, alt. 4000 m, Febr. 23, 1953. H. Sleumor (F), TYPUS.

Panellus mirabilis sp. nov.　Fig. 6.

Pileo albo, dein albo-alutaceo, saepe ochraceo-melleo-maculato in siccis, sub lente pubescente, dein glabrescente, levi, sed in vetustis plerumque margine lobulato-crenato vel sulcato, convexo, 1–4 mm lato. Lamellis pro ratione paucis, subdistantibus, applanatis, subangustis, haud venosis, acie integris, albidis acie demum

ochracentibus, adnatis. Stipite pileo concolori, pubescente, dein subpruinosulo in parte superiore, albo-lanoso tomenti mycelialis causa in parte inferiore, in primordio centrali, dein excentrico vel sublaterali, aequali, 1–4 mm longo, 0.3–0.4 mm lato. Carne alba vel albida, inodora. Sporis 6.3–10.3 × 2.8–3.6 μ, hyalinis, levibus, ellipsoideis vel anguste cylindraceis, fortiter amyloideis; basidiis 20.5–21.5 × 4.3 μ, clavatis, tetrasporis; basidiolis fusoideis, acuminatis; cystidiis in zona angusta ad et prope aciem numerosis, solitariis vel fasciculatis, 21 –25 × 6.3–10 μ, membrana nonnihil incrassata (usque ad 0.7 μ), clavatis, incrustatione abundante resinosa mellea vel ochracea instructis, ceterum hyalinis, demum pallide stramineis; epicute pilei hyphis fortiter diverticulatis (interdum aspectum asterostromelloideum fere
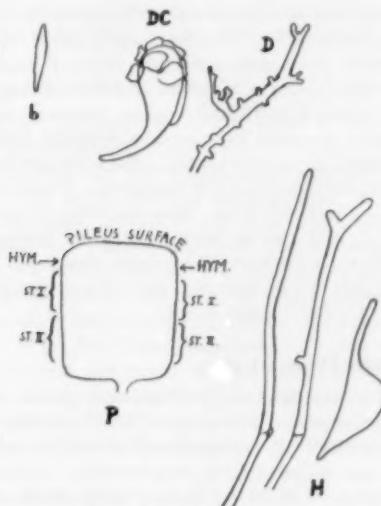

FIG. 6. *Panellus mirabilis.* *b.* Basidiole, × 500. *DC.* Dermatocystidium, × 1000. *D.* Diverticulate hyphal elements of the epicutis of the pileus, × 1000. *P.* Schematic longitudinal section through the primordium of 0.2 mm diameter, showing the origin of the hymenium (HYM) and the upper (ST.I) and lower (ST.II) zones of the stipe. *H.* Hairs of the covering layer of the lower zone of the stipe, × 500.

monstrantibus) formata et inter hyphas dermatocystidiis eis hymenii simillimis obsita; epicute partis superioris stipitis structura sua cum eo pilei identica; tegumento partis inferioris stipitis ex hyphis filamentosis pilosis mycelii formato; hyphis omnibus haud amyloideis, fibuligeris. Primordiis gymnocarpicis. Ad ramos emortuos in dumeto subtropicali uliginoso (low hammock) gregatim, Highlands Hammock State Park, Highlands Co., Florida, U.S.A., Aug. 12, 1942, Singer *F188* (ī), TYPUS.

Crinipellis foliicola sp. nov.

Pileo brunneo (inter "Prout's Brown" et "Mummy Brown" vel "Mummy Brown" R), hygrophano (statu sicco inter "Dresden Brown" et "Cinnamon Brown"

R), centro subumbilicato atrobrunneo, subappresse undulatopiloso interque fascicula pilosa trama pilei denudatum album monstrante, convexo dein plano, 5–6.5 mm lato. Lamellis albidis, mediocriter vel moderate confertis, latis (1–1.5 mm), abrupte rotundato-liberis. Stipite brunneo, basin versus atrobrunneo, hirsuto-piloso, aequali, insiticio, 15–19 × 0.3–0.8 mm. Carne alba, exigua, inodora. Sporis 7.2–8 × 3.5–4 μ, plus minusve bis longioribus quam latis, hyalinis, membrana subtenui instructis, levibus, ellipsoideis vel fusoideis; basidiis 22 × 5.5 μ, tetrasporis; basidiolis fusoideis; cystidiis nullis; cheilocystidiis (8–)14–22 × 3.7–5.7 μ, clavatis vel cylindraceis, interdum furcatis vel tripartitis, frequentius integris sed plerumque excrescentiis sterigmatoideis 1.5–3.8 μ longis stricte apicalibus instructis et tunc eis C. *diptero-carpi* analogis, hyalinis vel melleohyalinis, acie lamellarum heteromorphia; pilis zonae marginalis pilei apicem attenuatum sed obtusum versus frequenter sparse vel dense septatis (septis defibulatis), interdum septis nullis et rotundatis, rarius admodum acutis, umbrinis, sed apicem aut basin versus frequenter hyalinis, membrana crassa (1–1.5 μ diam.), stricta, undulata, interdum nodosa praeditis, longissimis, 3.7–5 μ diametro; strato hypotrichiali hyalino, elementis irregulariter dilatatis formato; hyphis tramatis regularis hymenophori fibulatis, hyalinis, inamyloideis; pilis omnibus plus minusve pseudoamyloideis. Ad folium putrescens *Similacis* sp. loco paludoso in dumeto subtropicali (low hammock), Highlands Hammock State Park, Highlands Co., Florida, U. S. A., Aug. 30, 1942, Singer *F444* (F), PARA-TYPUS. Ibidem, Sept. 2, 1942 (hic in foliis variis, haud tantum *Smilacis*), Singer *F444a* (F), TYPUS. In siccis centrum pilei minime obtuse papillatum haud nigrum sed manifeste obscurius coloratum, haud glaberrimum sed subglabrum apparet; zona centralis minutissima. C. *dipterocarpi* affinis.

Pseudohiatula panamensis sp. nov.

Pileo atrocinereo, centro fuligineo, per miximam partem radii rugoso-sulcato, apparenter subumbonato, convexo, dein repando, 22–35 mm lato. Lamellis in siccis sordide griseis, subdistantibus vel distantibus, intervenosis, intermixtis, latiusculis, adnexis, lineis venosis per apicem stipitis decurrentibus. Stipite pallide griseo aut albido-griseo, aequali tubuloso, 30–40 × 2–3 mm. Carne albida (?). Sporis 7.8–10.8 × (4.3–)5.3–7.8 μ, frequentissime 9.3 × 5.8 μ hyalinis, levibus, inamyloideis, tenuitunicatis, late ellipsoideis; basidiis 28.5–30 × 5–7.3 μ, tetrasporis; paucis bisporis; cystidiis paulum manifestis, haud opacis, e. gr. 35 × 8.5–12.5 μ, vesiculoso-clavatis vel clavatis, subbasidiomorphis, tenuitunicatis, haud numerosis, paulum projicientibus; epicute pilei e cellulis obpiriformibus vel subglobosis 23–43 × 18–24 μ, succo fusco impletis vel hyalinis epithelium formantibus consistente, dermatocystidiis subversiformibus, plerumque ampullaceis vel cylindraceis, in hypodermio natis, apice rotundato instructis, levibus nudisque, succo fusco impletis intermixta; hypodermio cutem formante, ex elementis elongatis maxima e parte fortiter pigmentatis pigmento fusco intracellulari dissoluto; tramate pilei ex elementis hyphosis hyalinis, haud gelatinosis inamyloideis, defibulatis consistente. Probabiliter ad lignum emortuum, in silva tropicali, Corundú Zona Canalis, Pana nae, Aug. 13, 1952, Martin & Weldon *8335* (F, IA) TYPUS et ISOTYPUS.

Marasmiellus defibulatus sp. nov.

Pileo albo in vivis siccisque, irregulariter neque profunde sulcato, sicco, subvelutino (sub lente), convexo, subumbilicato, 2.5–4 mm lato. Lamellis albis, adnatis,

subdistantibus, moderate latis vel sat latis. Stipite umbrino sed ad apicem albo, demum toto umbrino, subfibrilloso, insiticio, centrali sed fortiter curvato, 1.5–5 mm longo, medio millimetro tenuiore. Carne tenerrima, alba; odore nullo. Sporis 6.5–8.5 × 3.5–4 μ, hyalinis, levibus, interdum excrescentia subangulari praeditis vel duplicatis, sed plerumque simplicibus integrisque, ellipsoideis, ad latus internum frequenter subapplanatis, inamyloideis; basidiis 16–22 × 6–6.5 μ, clavatis, tetrasporis; cystidiis nullis; cellulis diverticulatis aciei lamellarum cheilocystidiiformibus, numerosissimis, elementis diverticulatis epicutis pilei analogis; epicute pilei ex hyphis nodosis vel ramosis, manifeste diverticulatis, hyalinis efformata, "structuram *Ramealium*" manifestante; tramate lamellarum regulari, hyalino, cum hyphis omnibus inamyloideo, ex hyphis filamentosis ita ut basis basidiorum fibulis destitutis efformato. Ad folium dejectum, 5 km occ. Opp. Paranaguá, Paraná, Brasiliae, in silva tropicali litorali, Sept. 28, 1952, Singer *B401* (F), TYPUS.

Marasmiellus septicoides sp. nov.

Pileo albo, glabro, per medium radium externum sulcato, ceterum levi, sicco, sub lente fibrillis arachnoideis appressis obtecto, convexo, 2–8 mm lato. Lamellis albis, latis, subdistantibus vel distantibus, liberis. Stipite albo, minuto, curvato, hymenophoro appresso, usque ad 3 × 1 mm; tomento basali nullo, basi insiticia. Carne subnulla; odore nullo. Sporis 11.5–12 × 4.5–5 μ, hyalinis, accumulatis pallidissime flavidulis, membrana subtenui instructis, inamyloideis, subfusoideis, latus internum versus applanatis; basidiis 30.5 × 8.2 μ, clavatis, hyalinis, paucis flavidulis, tetrasporis; cystidiis ad aciem numerosis, ad latera lamellarum sparsis, hyalinis, raro flavidulis, plerumque basi incrassata praeditis (6–7 μ diam.), ceterum filamentosis (4–4.5 μ diam.), e. gr. 35 μ longis; hyphis epicutis pilei tenuistratosis, appressis, hyalinis, elongatis, levibus, sed sparse ramulis nodulisque densiusculis instructis et admodum subtiliter neque manifestius diverticulatis; dermatocystidiis nullis, pilis nullis; hyphis inamyloideis, fibulatis. Ad ramum putridum in silva tropicali litorali, Angra dos Reis, Rio de Janeiro, Brasiliae, Oct. 1. 1952, Singer *B427* (F), TYPUS.

Marasmiellus echinocephalus sp. nov.

Pileo albido, centro fasciculo alto setarum erectarum penicillato, sparsius setoso marginem versus, interdum in zona marginali haud setoso, sicco, margine interdum subfimbriato, convexo, 3–6 mm lato. Lamellis cremeo-albis, saepe furcatis vel anastomosantibus, angustioribus, acie obtusis, subveniformibus, frequenter ad marginem pilei nullis, distantibus, decurrentibus. Stipite opace ochroleuco-albulo, subtiliter sericeo-subfibrilloso, basin versus manifeste pruinato-velutino, basin versus incrassato, insiticio, 3–8 × 0.4 mm. Carne alba. Sporis 12–17 × 4.3–5.7 μ, oblongis vel cylindraceis demumque curvatis *Fusarii* modo, hyalinis, levibus, inamyloideis; basidiis 25.5–29.5 × 7–9 μ tetrasporis, clavatis; cystidiis cheilocystidiisque nullis; epicute pilei cutem formante; eius hyphis levibus, appressis vel subascendentibus, hyalinis; setis erectis, deorsum geniculatis, ui apicem acutum vel subacutum attenuatis, fulvo-castaneis, obscurioribus basin versus, pigmento membranaceo, crasse tunicatis (membrana 2 μ crassa), levibus, inamyloideis, 150–200 μ longis; stipitis superficie hyphis hyalinis filamentosis composita, omnibus appressis parallelisque; ex eis hyphis nonnullis erectis emergentibus, hyalinis, levibus, integris, apice rotundatis; basi stipitis pilis numerosis fulvo-castaneis setis pilei analogis obsita, sed hic sunt breviores frequenterque ramosi ad apicem nodos exiguos vel appendicu-

lationes longiores gerentes; hyphis omnibus inamyloideis, fibulatis. In petiolis Palmae cuiusdam, Barro Colorado Island, Zona Canalis Panamae, Martin *4082* (F, 1A). TYPUS et ISOTYPUS. Hic fungus *M. candido* var. *setuloso* affinis.

Volvariella lepiotospora sp. nov.

Pileo brunneo-nigro in centro, ceterum fibrillis radiatim dispositis brunneo-nigris vel subnigris obtecto, subrimuloso vel subrimoso, centro integro, tota cuticula tegumento tenui tomentelloso pallide stramineo obtecto, convexo, dein applanato, umbonato, 32 mm lato. Lamellis roseis, acie roseis, fimbriatis, confertis, liberis. Stipite pallide sordideque grisello, subpruinato, apicem versus attenuato (usque ad 2 mm diam.), 50 × 5 mm; volva cortinoidea, arachnoidea, grisea. Sporis elongatis exiguisque, 4.7–5.5(–6.3) × 2.8–3.2(–3.8) μ, roseo-stramineis, levibus; cystidiis exiguis vesiculosis; cheilocystidiis clavatis, 21–26 × 7–9.6 μ; epicute pilei ex hyphis radiatim dispositis latiusculis, catenas formantibus, elongatis, raro subisodiametricis, succo fusco impletis, levibus, usque ad 30 μ latis formata; cellulis terminalibus apice
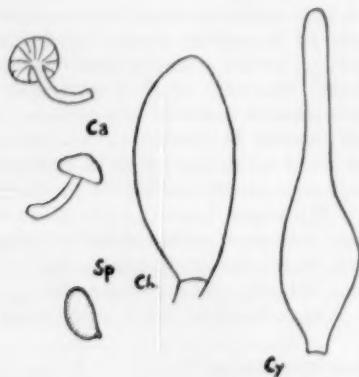
FIG. 7. *Pholiota naucorioides*. *Ca.* Carpophores, × 1. *Sp.* Spores, × 1000. *Ch.* Cheilocystidium, × 1000. *Cy.* Pleurocystidium, × 1000.

late rotundatis, 40–78 × 10–13 μ, brevioribus (raris) ± 80 × 47 μ; strato superficiali velari superposito ex hyphis tenuibus, hyalinis vel stramineo-hyalinis consistente; hyphis fibulatis. Ad radicem emortuam quercinam (*Quercus virginianae*) in dumeto tropicali, Matheson Hammock, Dade Co., Florida, U.S.A., Oct. 20, 1942, Singer *F1143* (F), TYPUS.

Pholiota naucorioides sp. nov. FIG. 7.

Pileo pallide stramineo, dilute ochraceo-maculato, maculis e fibrillis stratum superficialem, frequenter interruptum formantibus compositis, ita ut interdum superficies pilei subsquamulosa appareat, cuticula fundamentali subfibrillosa, haud viscida, levi, haud striato, dein in depressione centrali maculis accumulatis intensius colorato atque disco ferrugineo-ochraceo-brunneo, demum e sporis in superficie depositis saepe obscurius brunneo, convexo, leniter umbilicato, demum convexo centro depresso,

praesertim in siccis, 5–7 mm lato.   Lamellis pallide argillaceis (numquam flavidis),
demum obscurius argillaceo-brunneis, subinde subferruginascentibus, praesertim in
siccis, latis, late adnexis, vel adnatis, moderate confertis vel subconfertis; sporis in
massa subferrugineis.   Stipite pallido vel ochroleuco-stramineo-pallido, subtiliter
sericeo-fibrilloso et demum frequenter glabrescente, sicco, solido, aequali, plerumque
tamen curvato nec non aut apice aut basi aut plerumque basi apiceque incrassatis,
9–11 × 0.8 mm, apice aut basi aut ambobus usque ad 1.2–1.5 mm latis; velo fugaci,
ad marginem carpophororum juvenilium haud manifesto, pallido, numquam annu-
lato; tomento basali abundante, albo.   Carne pallida, exigua; odore nullo, sapore
haud amaro.   Sporis 7–8.2 × 4.3–5 μ (rarius maioribus, e basidiis bisporis natis:
9.8 × 5.5 μ), ellipsoideis vel cylindraceis vel frequentius ovoideis, duplice tunicatis,
levibus, apice haud truncatis sed interdum poro germinativo completo exiguo praedi-
tis, ferrugineo-ochraceo-brunneis, apice subacutis vel frequentius rotundatis, de-
pressione applanationeque suprahilaribus destitutis, numquam reniformibus; basidiis
21–22 × 7.5–8 μ, hyalinis vel rarius flavidis (e pigmento diffundente intracellulari),
tetrasporis, paucis bisporis; cystidiis ad aciem lateraque lamellarum numerosis sed
haud dense dispositis, incrustationibus nullis vel rarius hyaline vel flavide incrustatis,
corpusculo flavo interno refractivo destitutis (chrysocystidiis absentibus), ampul-
laceis, 40-55 × 9.5–10 μ, apice longo, 4.5–6 μ diam.; cheilocystidiis ampullaceis vel
subvesiculoso-ventricosis, hyalinis vel rarissime subflavidis, apice rotundatis, 30–33 ×
8–14.2 μ, interdum basidiis inter ea dispersis; tramate hymenophorali regulari, fila-
mentoso, hyalino, sed nonnullis elementis in eo et in subhymenio saepe flavido-
tinctis e pigmento intercellulari in ammonia dissolubili; epicute pilei e trichodermio
constante quod partim ad superficiem appressum est, ex hyphis catenulatis fortiter
pigmento ferrugineo-incrustatis, angustis filiformibus vel latis, septatis cum fibulis,
apicibus rotundatis formata; incrustationibus crystallinis nullis; supra tale stratum
velare atque inter fragmenta eius cuticula ipsa ex hyphis filamentosis repentibus
minus pigmentatis formata disposita est; hyphis omnibus fibulatis.   Ad truncum
arboris dicotyledoneae recenter caesae in silva tropicali litorali gregatim, prope
Paranaguá, Paraná, Brasiliae, Sept. 27, 1952, Singer *B410* (F), TYPUS.   Structura
strati velaris pilei ad *Phaeomarasmium* tendet, at cystidiis recedit; *Pholiota* flammu-
loides est staturaque minuta naucorioides.

Crepidotus martini sp. nov.

Pileo brunneolo-albo, levi, convexo, subtiliter pubescente vel subglabro, 2–4 mm
lato.   Lamellis brunneis, subdistantibus, pro ratione obtusis in acie dum juveniles
sint, moderate latis, liberis vel adnatis; sporis in massa concoloribus.   Stipite
primum (in primordio) centrali, dein excentrico vel sublaterali, mox oblitterato, in
maturis interdum absente, minuto, curvato.   Carne albida.   Sporis 6.3–9 × 4.7–5.3 μ,
plerumque 6.5–7.5 × c.5 μ (expraeparatione sporarum, omnium maturarum), levibus,
melleis, unicoloribus, ellipsoideis, rarissime depressione suprahilari gaudentibus epi-
sporio vix manifeste punctato sed leniter heterogeneo spinulis paulum differentiatis
immersis *C. luteoli* modo; basidiis 17–19 × 6.8–7.2 μ, tetrasporis; cystidiis nullis;
cheilocystidiis 19–30 × 7.8–13.5 μ, numerosis, sed interdum basidiis interruptis,
admodum versiformibus, frequenter vesiculosis, saepe ventricoso-subcylindraceis et
centro leniter constrictis, rarius apicem versus attenuatis vel clavatis; epicute pilei
et hyphis repentibus filamentosis formata, cellulis terminalibus elongatis apicibusque
rotundatis, hyalinis vel melleis, sed pigmento incrustante destitutis, 4–4.5 μ latis;

hyphis omnibus fibulatis. Ad quisquilias, ramulos dejectos, stipites foliaque emortua abundanter gregarius, Barro Colorado Island, Zona Canalis Panamae, Jul. 7, 1952, Martin & Welden *7494* (F, IA), TYPUS et ISOTYPUS. *Crepidoto peziculae* (Berk. & Br.) Sacc. ceylonico peraffinis, sed hic hyphas epicuticulares pigmento ferrugineo incrustatas habet.

Pleurotellus fibulatus sp. nov.

Pileo albo, glabro, ceterum omnino ut in *P. herbarum*. Lamellis albo-cremeis, confertis vel subconfertis, adnexis vel decurrentibus, vix latis. Stipite minuto, laterali vel nullo. Carne alba. Sporis 7.8–9.2 × 3.5–4.7 μ, levibus, teretibus, melleis, membrana tenuicula haud manifeste duplici instructis, callo poroque destitutis, membrana homogenea praeditis; basidiis 18–27 × 4.7–5.7 μ, tetrasporis; cystidiis nullis;
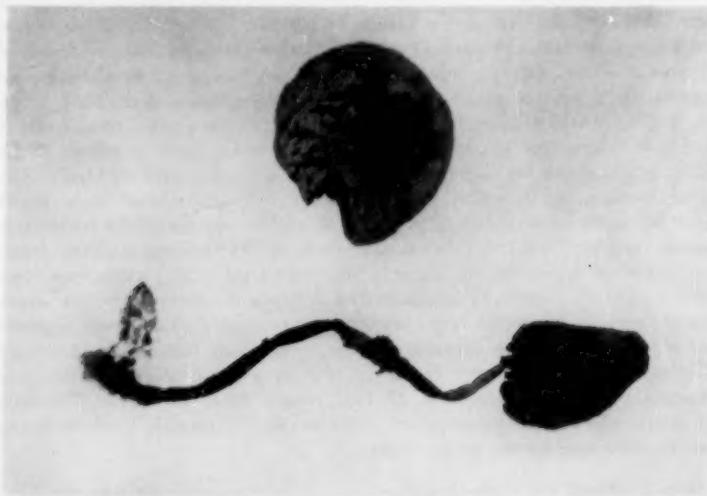

FIG. 8. *Brauniella alba*. Carpophores, natural size, phot. Singer.

cheilocystidiis 8.5–10 μ latis, sparsis, ventricosis, longitudine basidia aequantibus; hyphis fibulatis. Ad Montem Alsea, Benton Co., Oregon, U.S.A., Doty *723a*. Nov. 4, 1939 (F), TYPUS. *P. herbarum* affinis sed fibulis differt.

Brauniella Rick ex Sing. nom. nov.

Braunia Rick, Egatea **19**: 112.   1934.

Brauniella alba (Rick, Egatea **19**: 112.   1934) Rick ex Sing. comb. nov. FIG. 8.

This species was studied and redescribed in Lilloa **26**: 102.   1953, as *Braunia alba* Rick.

It was then indicated that Rick had used a herbarium and manuscript name *Brauniella* for this interesting new genus, since he realized that *Braunia* was preoccupied. As I have explained in footnote (1) in the Lilloa paper, his assumption that *Braunea* is a homonym of *Braunia* is wrong. However, Rick's genus is a homonym of *Braunia* Bryol. Eur. 1846 (Hedwigiaceae), and consequently Rick's alternative unpublished proposal to rename the Gastromyete *Brauniella* has been accepted in a recent communication made by the present author at the VIII International Botanical Congress at Paris, 1954.

Brauniella alba (Rick) Rick ex Sing. is related to the *Pluteeae* (fam. *Amanitaceae*) among the Agaricales, and is similar in certain aspects to *Volvariella cnemidophora* (Mont.) Sing. Among the Gastromycetes, it belongs in the family *Secotiaceae*. It is also related to the genus *Torrendia* Bres.

Material from the state of Rio Grande do Sul, Brazil, preserved at the Herbarium of Collegio Anchieta, Pôrto Alegre, Brazil.

Fundación Miguel Lillo
Tucumán, R. Argentina

REVIEWS

PHYTOPATHOLOGIE DES PAYS CHAUDS. Vol. III, by L. Roger (Encyclopedie Mycologique, Vol. XIX). Pp. 2257–3154, figs. 362–368. Paul Lechevalier, Paris, 1954. Unbound. Price 10,000 francs (about $28.00).

Vols. I and II of this work have been noted in Mycologia **44**: 424 and **45**: 625. The third, and concluding, volume deals with diseases due to algae, mosses and lichens (25 pp.), bacteria (139 pp.), phanerogamic parasites (32 pp.) and viruses (175 pp.). The remainder of the volume is devoted to the third part "Revue des problèmes phytosanitaires des pays chauds" arranged by hosts or host groups, and followed by a glossary, tables of contents and two complete indexes to the entire work, one to names of organisms and topics treated; the other to diseases and causal organisms classified by hosts. The general treatment and the indexes greatly facilitate the usefulness of the work for reference. As stated previously, the encyclopedic nature of this series makes it a necessity for all laboratories of plant pathology, since many of the diseases discussed are common in temperate areas.—G. W. M.

A MONOGRAPH OF THE FUNGUS GENUS CERCOSPORA, by Charles Chupp. 667 pp., 222 figs. Published by the author, Department of Plant Pathology, Cornell University, Ithaca, N. Y. 1953. Price $10.00.

This very large parasitic genus, including many common and widely distributed species, has long been in need of monographic treatment. This is presented, on a world-wide basis, in the volume under consideration.

A brief introduction gives the history and characteristics of the genus and comments on the various attempts that have been made to erect segregate genera for various groups of species. The author concludes that none of these are practicable. The chapter concludes with a key to 40 genera which have been confused with *Cercospora*.

The over 1200 recognized species, some of which are here described for the first time, are arranged by host families, the latter listed alphabetically. There is no general key, but throughout the text, there are numerous keys to the species occurring on specific families or genera. An index of host names and another index of recognized species and synonyms completes the volume. The illustrations are simple line drawings showing spore characters, conidiophores and outlines of stromata, placed in the text in convenient proximity to the species illustrated—about a fifth of the total.

The book is obviously needed for reference in all plant pathology and mycology laboratories.—G. W. M.

MANUSCRIPT

Publication in MYCOLOGIA is ordinarily restricted to those who have been members in good standing of the Mycological Society of America for over a year immediately preceding submission of manuscript. Exceptions to this regulation require a favorable vote by a majority of the Editorial Board. When a paper has two or more authors, the person submitting the paper is expected to be a member.

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